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The Action of the Thyroid Gland in the Induction of Experimental Medial Arteriopathy

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APPROVAL SHEET

The dissertation submitted by Adolph P. Reszkowski has been read and approved by five members of the faculty of the Graduate School.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form, and mechanical accuracy.

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

31 May 54
Date

H. T. Butler
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LIFE

Adolph P. Roszkowski was born in Chicago, Illinois, July 27, 1928.

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CHAPTER I

INTRODUCTION

CLASSIFICATION OF ARTERIOPATHIES

The term arteriosclerosis means, literally, "hardening of the arteries" (gr. skleros= hard). It is a generic term. It includes various types of degenerative and morbid vascular processes. The human being, as well as other animals, is subject to these various vascular derangements. It would be well at present to consider the various types of known arteriopathies. The nomenclature committee of the American Society for the Study of Arteriosclerosis ('53) has reported a tentative classification of arteriopathies. A brief resume of this report will help facilitate an understanding of the study undertaken for this dissertation.

I. Degenerative Arteriopathies

A. Atherosclerosis.

This disease is exemplified by an intimal proliferation displayed by plaque-like deposits of neutral fats and lipophages. Lesions tend to develop, become fibrotic and calcification may take place. The lumen of the vessel may be encroached upon by these lesions and thrombi, therefrom, lead to vascular occlusion.

B. Medial Arteriosclerosis (Moenckeberg's Arteriosclerosis).

This disease is characterized by profuse deposition of calcium and fibrotic tissue in the media. This leads to dissecting aneurysms and aortic insufficiency. This disease is usually associated with the aged, although occasionally young individuals are afflicted.

C. Arterionecrosis.

This is a disease involving either a cystic degeneration of the medial coat or a necrosis of the blood vessels due to various toxic substances.

II. Productive or Hyperplastic Arteriopathies

A disease involving small arteries or arterioles displaying hyperplasia of the media. This is apparently due to an increased arterial pressure.

III. Inflammatory Arteriopathies

A number and variety of arteriopathies occur which may be the result of:

1. Infectious micro-organisms
2. Hypersensitivity reactions
3. Toxic chemical agents
4. Physical factors
5. Mechanical trauma
6. Undertermined factors

IV. Combined Forms of Arteriopathies

Many individuals display a combination of arteriosclerotic lesions. That is, an intimal proliferated area with atheroma superimposed upon an area showing medial lesions.

Most individuals investigating vascular abnormalities agree that the human is susceptible most frequently to atherosclerosis (Katz & Stamler '53; Hueper '44). Of primary concern is the fact that this vascular abnormality very often effects the coronary arteries. The intimal proliferation, intracellular and extracellular lipid accumulation, fibrosis, hyalinization and calcification may lead to occlusion of the coronary vessels, predisposing to myocardial ischemia and also infarction. Because of the widespread distribution of this arteriopathy in the human it has received a tremendous amount of attention. Needless to say, most investigators concerned with degenerative arteriopathies have centered their interest almost exclusively to this type of vascular derangement. Recent investigations of arteriopathies other than atherosclerosis have for the most part been infrequent although the older literature is abundant in its consideration of all types of vascular abnormalities (Hueper, '44; Allbutt, '15).

This dissertation deals with an experimental arteriopathy which is morphologically similar to a human medial arteriosclerosis of the Monckeberg type or a medial arterionecrosis. The arterial tunic most conspicuously involved is the media. Intimal proliferation is minimal or absent, there being no fatty or cholesterol infiltration associated with the vascular lesions. A method commonly employed in producing these lesions is that of Mikulicich and Oester ('52) which involves a daily administration of epinephrine and thyroxine over a several week period. The experimental arteriopathy thus produced is spectacular in that in a matter of a two and one-half weeks or less an experimental animal may display an extremely pronounced arteriopathy. The tunica media is

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primarily involved. There is a derangement of the elastic and muscular fibers. Generally this involved area becomes calcified and in animals displaying a severe arteriopathy the entire vessel (thoracic aorta) may become infiltrated with calcium deposits.

The relation of this experimental epinephrine - thyroxine induced medial arteriopathy to any known human vascular derangement is problematical and not within the scope of this dissertation. It is of interest to note that various investigators (Lansing '52) maintain that medial arteriosclerosis and atherosclerosis are intimately associated.

The etiology of the degenerative arteriopathies for the most part is not well understood. Because of this, a review of selected pertinent experimental and observed clinical findings will help to clarify the description of various arteriopathies.

THEORIES OF ARTERIOSCLEROSIS

Theories of arteriosclerosis are manifold. In a consideration of any theory proposing to explain the genesis of arteriosclerosis a realization of the specific type of vascular abnormality one is dealing with is essential for the explanation. Thus, probably no one theory is adequate in explaining all of the known human vascular abnormalities. Yet, as will be shown, "universal theories" of arteriosclerosis have been purported. Some of the following are of that nature.

A. The "Aging" or Senescence Theory

The thesis of this theory is simple. It merely states that arteriosclerosis is an inevitable process occurring in animals as a result of increase in age. Much evidence may be cited along these lines to indicate

a positive correlation between incidence of arteriosclerosis and increase in age. Hueck ('20) made the observation that persons between the ages of 60 and 70 showed fatty, calcium and hyalin depositions in vascular walls. Ophuls ('33) reported the incidence of arteriosclerosis according to the following age scale: 20-29 years, 3.5 per cent; 30-39 years, 9.25 per cent; 40-49 years, 26 per cent; 50-59 years, 48 per cent; 60-69 years, 78 per cent; greater than 70 years, 90 per cent. More recently, Yater et al ('51) described a similar positive correlation between atherosclerosis and increase in age.

Wells ('33) explains this phenomenon on the basis that the medial collagenous and elastic components of arteries undergo an irreversible hydration and thickening with age. This is explained purely on a physical basis, the author contending that the media reacts comparable to an old worn out automobile tire.

With the advent and general acceptance of the hypercholesterolemia theory the senescence theory has largely lost favor. Recently, however, Lansing et al ('60) have proposed a modern counterpart to the senescence theory.

Arguments against the validity of the senescence theory are very numerous. Huser ('44) points out that arteriosclerosis is a subtle, slowly progressive, chronic vascular alteration. Because of this slow change, the pathological manifestations cannot and usually do not become apparent until later years of life. This indicates that though these vascular aberrations are more numerous with increased years, the disease condition may have been initiated relatively early in life. It should also be pointed out that arteriopathies may occur in infants (Hause and Antell '47) or in young

individuals (French & Dock '44, Newman '46, Brandt '50, Adlersberg and Zak '50). On the other hand, older individuals are frequently free of arteriopathies at autopsy (White et al '50, Howell & Piggett '51). This indicates that arteriosclerosis is not necessarily and inevitably a consequence of aging.

Another obvious facet of arteriosclerogenesis incompatible with the senescence theory is the uneven distribution of the arteriopathic lesions along the vascular tree. Certainly if arteriosclerosis were merely a matter of simple aging, how could one explain the relatively high incidence of arteriopathies along the coronary, cerebral, aorta, pulmonary and renal vessels but not along the large extremity and peripheral somatic vessels? It is also noteworthy that arteriosclerosis of the coronary vessels is quite prevalent in the middle-aged male human but usually low in incidence in the comparable aged human female. Clawson & Bell ('49) reported a tenfold increment of fatal coronary artery disease in the human male as compared to the female in an age group of less than 40.

From these facts and many other similar studies, workers in this field generally conclude that arteriosclerosis, including the various degenerative arteriopathies, is not an inevitable consequence of aging. In addition a large mass of positive evidence, yet to be discussed, indicates a more probable causative factor of arteriosclerogenesis.

B. The Mechanical Trauma Theory

The view that actual physical or other traumatic injury is totally or in part responsible for a vascular damage leading to or predisposing to various types of chronic degenerative arteriopathy is considered likely by

a large number of investigations (Allbutt '13, Duff '35, Taylor '55). Thomas ('53) made the interesting observation that arteries whose medial coat was weakened or impaired as to elasticity displayed a compensatory intimal proliferation. In effect, he concluded that a medially weakened artery bulges because the damaged media does not allow for proper maintenance of arterial "tonus". At this site the caliber of the vascular lumen is increased. A compensatory mechanism of such a blood vessel is an intimal proliferation. This observation has been verified many times. Some of the observed and experimental findings which substantiate this concept are:

1. Electric injury - Lange ('24) was able to induce medial necrosis of carotid arteries upon prolonged electrical stimulation at this area.

2. Irradiation injury - Warren ('42) has reviewed the effect of ionizing radiations on the vascular tree. Relatively small amounts are needed to damage small vessels. It appears that small vessels whose principle component is endothelium are most prone to irradiation injury. The medial components are effected only by irradiation doses of 500 r. or more.

3. Injury due to heat or cold - the effect of cautery has been studied by Szolowjew ('30). It was observed that the integrity of the medial elastic components must be maintained if proper regeneration is to be achieved. If the medial elastic fibers are destroyed, the entire area will be replaced with granulation tissue.

Taylor ('50) employed an ingenious CO₂ freezing needle device, which he inserted into the arterial wall and caused damage to the surrounding area. Aneurysmal dilatation occurred in the damaged area because of muscle tissue damage. Following this there occurred a pronounced intimal prolifera-

tion.

4. Mechanical Trauma - experimental studies concerned with mechanical injury to blood vessels are quite numerous. The effects of crushing, adventitial damage, etc. have been reviewed by Anitschkow ('33). The pathology and the repair process displayed by these procedures are similar to those occurring from medial damage due to previously mentioned experimental procedures.

5. Injury due to abnormal pressures on general body surfaces - When increase or decreased gravitational forces (G forces) are exposed to body surfaces the arteries of the body are subjected to abnormal tensile forces (Stapp '51; Shaw '48). Intracranial hemorrhages have been induced in experimental animals due to abnormal gravitational forces. These are probably due to direct tearing of such blood vessels.

The many findings reported on the role of trauma in the induction of arteriosclerosis are not considered as being truly significant by the most prolific workers in the general field of arteriosclerosis (Kusper '44; Katz & Stamler '53). Nevertheless, theories have been proposed which incorporate these findings as well as various others into a broader concept of arteriosclerogenesis. These views will be discussed in a subsequent portion of this dissertation.

C. The Toxin Theory

The idea that toxins of bacterial or other origin and also other cytotoxic substances are important in aortic sclerogenesis has been advocated by many investigators. A rather large number of clinical studies have indicated that an infectious disease state renders an individual prone to aortic

inflammations (Ophule '21). Thus, Faber ('24) noted that sclerotic arteries were closely associated with the tuberculosis state. Chronic disease such as syphilis, rheumatic fever and heart disease are associated with severe types of arteriopathies (Anitschkow '25, Pappenheimer & Van Olahn '24).

Experimental evidence favoring the toxin theory of arteriosclerosis is rather sketchy. Duff ('32) was able to induce an arteriopathy in which intimal and medial changes took place in the arteries of the spleen, liver, lungs and kidneys by means of injection of diphtheria toxin into vitamin C deficient guinea pigs. Menten and King ('35) were able to induce a similar arteriopathy by injecting Pneumococci, Staphylococci, B. coli, B. typhosus, Salmonella enteritidis, and Streptococci toxins into guinea pigs deficient in vitamin C.

Other investigations have not been as conclusive as the above in that injections of bacteria do not as a rule induce arteriopathy. Mac Callum ('33) with a formidable array of clinical and experimental evidence came to the conclusion that infectious disease states for the most part, are not significant factors in the etiology of arteriosclerosis.

From the preceding evidence and because of the fact that medial and intimal arteriopathies may be produced experimentally in a more "physiologic" manner, the toxin theory is not championed nor advocated by many.

D. The Hypoxia Theory

A great variety of substances are able to induce sclerotic lesions of blood vessels. Many of these substances are of endogenous origin - hormones, amino acids, metabolites, etc. A whole host of exogenous materials are also able to produce arteriopathies - heavy metal ions, vasotonic drugs

and other substances. In addition the previously mentioned substances also have to be considered as arteriosclerogenic in a broad sense.

In sclerogenesis, it is not conceivable, according to adherents of the hypoxia theory, that a separate, distinct mechanism is involved with each of the groups of substances mentioned above. Rather, a similar underlying factor is responsible for arteriopathy (Huesper '44). This "common denominator" of arteriosclerogenesis appears to be an anoxia, or more correctly a hypoxia, of the cellular components of the vascular wall. This anoxia would have to be selective in that the hypoxia state would predispose components in specific mural coats. Argued to the end, exponents of this theory would be led to the revelation that atherosclerosis would have as an essential etiologic episode a hypoxic state of the intima while the medial arteriopathies would develop as a result of a hypoxic medial condition, etc.

Now the means by which the hypoxic state was instituted would differ possibly according to the following plan:

I. Alteration of Vascular Tonicity

A. Hypotonia predisposing to arteriopathy.

Hypotensive agents such as nitroglycerin (Rabinowitch '44), aromatic hydrocarbons (Hayhurst and Meiswender '31) and other hypotonic agents if ingested chronically or acutely in large doses produce a vasodilation which may lead to peripheral dilatation causing a stagnant anoxia. Such a condition leads to medial necrosis and a calcification.

B. Hypertonia predisposing to arteriopathy.

Hypertensive agents of endogenous origin such as epinephrine (Josue '03) or other sympathomimetic amines (Loewi and Mayer '06) as well as adrenal

corticoids and renin (Masson '42) may initiate medial changes in renal and other vascular beds.

Exogenous chemical agents such as nicotine (Jesse '03, Thienes and Butt '38), barium chloride (Benecke '08) and other vasopressor agents induce an arteriopathy similar to the lesion appearing as a result of chronic epinephrine administrations. The anemia produced by the above and other vasopressor agents is of the constrictive type. That is, a transient or chronic hypertensive state is induced causing a constriction of the vascular lumen with a resultant lessened blood flow to the potentially affected vessel as well as the tributary vessels: such as the vasa vasorum in respect to the aorta. The resultant anemia affects primarily the medial muscular and elastic components. As a consequence, according to this theory, these components display the resultant lesions.

II. Alterations of Intravascular Pressure

The proper maintenance of intravascular hydrostatic pressure appears to be necessary for preservation of vascular integrity. Thus if the blood supply of a functional vessel suddenly ceases the vessel undergoes atrophic changes. An example of such changes have been observed by Bell ('33). This investigator noted a fibrous proliferation of the intima and media with subsequent degeneration of muscle cells in the human ductus arteriosus, postnatally. Similar changes can be observed in the umbilical arteries under these conditions. Experimental studies including the above principle were carried out by Malyschew ('29). This investigator double ligated a dog carotid artery from which blood was excluded. He observed high intimal mitotic activity and subsequent intimal proliferation. This was considered

to be a beginning necrotic, atrophic state. In all of these studies it may be concluded that an intimal hypoxia prevails due to inadequate oxygenation of the intima by intravascular blood. The media is also affected because of collapse of subsidiary vessels comparable to the vasa vasorum (Husper '45).

An excessive blood supply may possibly also affect vascular integrity. It is a well established fact that individuals in which systemic hypertension is manifest are more susceptible to a arteriosclerotic condition than are normotensive individuals (Daley et al. '43). In hypertensive individuals, on the other hand, large arteries having a relatively low intravascular pressure are remarkably free of lesions. A good example of this occurs in the pulmonary artery (Wilens '51).

According to exponents of the hypoxia theory the arteriosclerosis associated with increased intravascular hydrostatic pressure is also due to a hypoxic state. Thus in the hypertensive state the muscular and elastic components of the vessels are stretched beyond their normal range. In addition there is a compression of all the functional mural components against the rigid adventitia with the result that tributary vegetative blood vessels are compressed and a resultant ischemic hypoxia ensues. The vascular tissue compensates for this insult in the form of degenerative changes (Husper '45).

III. Alterations of Vascular Permeability

Many chemical agents such as methyl cellulose, polyvinyl alcohol, pectin, and other colloidal agents appear to be potent arterial sclerogens. Husper ('39, '41) upon chronic administration of polyvinyl alcohol to rabbits

and dogs was able to induce polyvinyl atheromatous changes in the lung vessels of rabbits and in larger coronary vessels as well as the aorta of the dog. A similar type of experiment was also performed by Hueper in 1942. This investigator injected methyl cellulose intravenously into rabbits and dogs. Acute and chronic observations were made. This worker observed what he considered a methyl cellulose atheromatosis of the aorta and other vessels. From sudan III staining of these atheromatous vessels the author concluded that no fatty infiltration occurred and that the intimal and medial cells affected were infiltrated with methyl cellulose. It was also shown by this investigator that pectin ('42), glycogen ('43) and other macromolecular colloidal substances induced atheromatous lesions comparable to those previously mentioned.

From this information Hueper came to the conclusion that these substances act as cellular films and coat the inner or endothelial layers of arteries. In so doing, they prevent the normal passage of oxygen from the blood to the inner cells of the blood vessels. This induces a hypoxia resulting in intimal proliferative and degenerative changes. Hueper ('45) also comes to the conclusion that cholesterol acts in the same manner. He points out that cholesterol is not readily subject to enzymatic breakdown. He therefore supposes that this substance may, by an alteration of blood solubility, come out of solution to form a macromolecular colloid; coat vessels and in consequence cause a hypoxia.

In this connection it should be pointed out that Lautsch, McMillan and Duff ('54) have employed methyl cellulose and thorium dioxide in colloidal form to induce atheromatous changes in rabbits apparently similar to those observed by Hueper. These investigators, however, demonstrated a hyper-

cholesterolemia as a result of injection of these compounds. The question therefore arises as to whether the atheromatous changes observed were a result of the colloidal compounds themselves or of the hypercholesterolemia which they induce.

B. The Hypercholesterolemia and Hyperlipemia Theory.

In 1933 Anitschkow reported that it was possible to produce atheromatous aortic changes in the rabbit by feeding this animal pure cholesterol along with its normal rations. The lesions induced were shown to be quite similar to those observed in the human. Since that time a tremendous amount of work has been performed with cholesterol induced atherosclerosis. As a result of this impetus, a popular theory of atherogenesis has evolved in which cholesterol is considered to be the primary causative atherogenic factor. There are several theories in turn defining the atherogenic action of cholesterol. Thus:

1. Anemia Theory of Haeper
2. The Filtration Concept - according to Page ('53)

Following this concept, atherogenesis is due to an accumulation of substances filtered by the lateral pressure from the plasma through the intima. Normally most of these substances pass thru the vessel harmlessly. However, abnormally some of the substances may remain within the mural tissue and in so doing set up a reaction. The inference is made by Page in that the filtrable substances are cholesterol fat or lipoproteins. The abnormal effect of these substances on the vascular constituents has as yet not been defined.

Now the factors which may induce an abnormal arterial response are very numerous. Because of the voluminous amount of work performed, only

mention can be made of some of the significant achievements.

3. Diet - Keyes et al. ('50) considers diet as being significant in arteriogenesis. A high cholesterol diet has been shown to be positively correlated with susceptibility towards atherosclerotic development. This worker also indicates that rigid adherence to a low cholesterol diet will significantly lower human hypercholesterolemia.

4. Hormones - Pick et al. ('52) have pointed out that coronary atherosclerosis is significantly lowered by estrogens in chicks placed on a cholesterol diet which normally induces coronary lesions. This is in general agreement with the observation that the human male is generally more prone to develop coronary heart disease than is the female.

Katz ('53) also has pointed out that thyroid administration may significantly lower experimentally induced atherosclerosis.

Other hormones also appear to be important in arteriosclerogenesis. As an example it has been indicated that the diabetic state greatly enhances the atherosclerotic state (Haist '49). Although many experimental studies have been performed involving endocrine glands and hormones other than the female sex and thyroid gland, the status of these substances has not been clearly established (Katz '53).

5. Species and Genetic Factors - Most experimentors in this field are of the opinion that the rabbit is the experimental animal most susceptible to arteriopathy. In addition the atherosclerotic condition can be induced experimentally in the chick, dog, guinea pig and duck. Rats, mice and other laboratory mammals are apparently free of atherosclerotic influences or develop lesions only under severe experimental influences. Experimental

medial arteriosclerosis has been induced in dogs (Waters '45).

That heredity is of importance has been pointed out by Adlersberg et al. ('49). It was pointed out that Jewish people were more susceptible to disturbed lipid metabolism or hypercholesterolemia (21%) than were non-Jews (9%).

6. Lipid and Lipoproteins in Atherosclerosis - Practically all of the cholesterol, neutral fat and phospholipid in blood is in combination with protein. Different amounts of these lipids are associated with alpha and beta globulin and alpha and beta lipoprotein. Barr and his associates ('53) using Cohn's method of fractionation have indicated that in conditions favoring atherosclerosis or in the atherosclerotic condition, the beta lipoproteins attain a high cholesterol level. Concomitantly, the alpha lipoproteins are relatively low in cholesterol content (Barr '53). Essentially the same conclusions have been reached in employing the ultracentrifuge method as described by Gofman et al. ('52).

As indicated by these investigations one of greatest stimuli to investigation in the field deals with the cholesterol concept of arteriosclerosis. Many workers would even go as far as to say that arteriopathies other than atherosclerosis, though they exist, are not of any consequence in the human when compared to atherosclerosis (Katz '53).

In this connection it might be well to indicate that several experiments demonstrate that a medial arteriopathy may set as a substratum or predisposing factor for what is generally considered to be human atherosclerosis. Thus, Hirsch and Weinhouse ('43) indicate that tissue factors may predetermine the site of an atherosclerotic lesion. They

point out that though the entire arterial tree is subject to the same blood cholesterol levels, nevertheless atherosclerotic lesions are predominantly found in the aorta and immediate branches. Thus, they conclude that vessels and tissues therein which are subject to unusual stresses are those rendered atherosclerotic. Anitschkow ('33) was also of the same opinion. Anitschkow ('14) and later Danisch ('28) showed that rabbits in which medial arteriosclerosis was induced by epinephrine or nicotine when subjected to a high cholesterol intake developed atherosclerosis changes which were directly superimposed over the areas in the vascular wall having medial tissue damage.

Duff ('35) in reviewing the above and similar experiments was especially impressed with the fact that cholesterol lesions are induced much more rapidly when the blood vessel has been previously injured. He points out that the blood cholesterol condition may be suitable for the development of atheromatous lesions without any such events occurring in normal blood vessels. This investigator therefore concludes that...."lipoid deposits in the arteries are always preceded by preliminary local alterations in the walls of the vessels which are indispensable to the formation of lipid accumulations, and which therefore constitute the initial stage of the development of experimental cholesterol arteriosclerosis."

More recently Kelly et al. ('52) showed that atherosclerotic lesions were superimposed on areas of the artery showing medial damage. The medial damage was induced by mechanically injuring the blood vessel by freezing. de Suto - Nagy and Waters ('51) were able to induce coronary atherosclerosis by the use of allyl-amine and hyper-cholesterol blood

levels. The lesions appeared very rapidly-in a matter of a few days or weeks.

In the view of these experiments the necessity for investigating all types of arteriopathies appears obvious. The ideas concerned with future paths of investigation have been aptly considered in this respect by Lehninger ('55) at the National Academy of Sciences and National Research Council symposium on Atherosclerosis who stated, ... "even the staunchest proponent of the 'lipoid' or 'cholesterol' theory present at this meeting will have to agree that there is no definite proof that a defect in lipid metabolism or transport is the primary event, and will have to agree also that local factors must play an important role because of the characteristically patchy distribution of the lesions."

EPINEPHRINE - THYROXINE INDUCED MEDIAL ARTERIOSCLEROSIS

1. Epinephrine as a Sclerogenic Agent

Epinephrine was first used to produce arteriosclerosis by Josue ('03). This investigator was able to induce these lesions in the rabbit by means of repeated injections of what was probably a crude preparation of epinephrine. Using animals greater than two kg., doses of approximately 200 micrograms were given per rabbit on approximately alternate days. Utilizing such a procedure, Josue was able to induce medial necrosis and calcification in the thoracic aorta. This was accomplished with as few as 5 such injections completed in five weeks and as many as 20 injections completed in three months. Only the results procured from three animals were presented in this report.

From this experiment Josue concluded that the arteriosclerosis so induced was the result of hypertension or hypertensive episodes produced

by the epinephrine. This investigator concluded that the aortic lesions he produced were similar to those observed in humans. In support for his contention he indicated that the experimental lesions were calcified, displayed aneurysms and contained blood pigments at the necrotic areas. No mention was made of the intima or of fatty accumulations at this site.

This discovery of Josue's was verified subsequently by many investigators. Bayloc and Albaredo ('04) were able to confirm the results of Josue. These investigators modified the dose of epinephrine employed as well as the length of the experiments. Using four rabbits, an interval of 22 days appeared to be adequate for epinephrine arteriosclerosis production..

Pic and Bonnamour ('05) observed that heavier (or older) animals are more prone to develop epinephrine arteriosclerosis than very young rabbits. These workers were able to produce these lesions with simple aqueous adrenal extracts showing that the induced lesions were not due to artifacts within the crude epinephrine of that day. These investigators were also able to demonstrate that the debilitated animal or animal whose resistance is lowered is more susceptible to develop these lesions than is a healthy one.

a. Histological Studies

Xrb ('05) made a very comprehensive study of epinephrine arteriosclerosis. He described both the gross anatomical and histopathological character of this degenerative process. He came to the conclusion that the epinephrine acts to destroy the character of the aortic tunica media with the result that there is a necrosis of the muscular and elastic elements of the media. These disrupted areas are secondarily replaced

by calcium. In some of Erb's chronic studies this investigator reports a thickening or proliferation of the tunica intima. In a number of animals Erb also found necrotic changes of the brachial, carotid and renal arteries. This investigator was of the opinion that this occurred as a consequence of vasa vasorum disturbance. Although Erb primarily employed the rabbit as an experimental animal, this investigator attempted to induce epinephrine lesions in the monkey (*Rhesus macacus*). Using two animals, no gross or microscopic lesions were observed to develop after 30 or 35 days respectively.

Arteriosclerotic involvement of vessels other than the aorta as described by Erb were not verified by Pearce and Stanton ('06). These workers investigated the early changes observed in blood vessels following epinephrine treatment. The earliest changes observed occurred after 5 days of treatment. Degenerative foci could be seen arising within the media. In animals treated for longer periods of time the necrotic areas of the media are replaced by calcium, this being the most conspicuous change. Intimal proliferative changes were also observed. These were reported to occur in animals after treatment of 24 days followed by a non-treatment period of 10 to 15 days. The intimal lesions were observed only over areas showing medial damage. This was reported as being a compensatory proliferation in an endeavor to strengthen the damaged vessel wall.

A more basic histological study involving the components of the media was undertaken by Scheidemandel ('05). This investigator was of the opinion that epinephrine effects primarily the elastic components of the

media. There is first a narrowing of the elastic lamellae followed by a granulation of the elastic components.

In general disagreement with the above opinions are the findings of Klotz ('06) who contends that the muscle cells are primarily the ones undergoing degeneration and necrosis. The muscle cells first undergo a fatty degeneration and this is followed by secondary deposition of calcium. The elastic fibers are only secondarily affected but when calcified, display the bulk of calcareous deposits.

This investigator further points out that epinephrine medial arteriosclerosis is unlike human atherosclerosis. He rather likens the medial arteriosclerosis to that observed by Moenckeberg ('03). He explains that the intimal proliferation observed by previous workers are incidental and are associated with underlying medial degeneration.

Ziegler ('05) and later Pearce and Baldauf ('06) were of the opinion that the aortic medial derangements due to epinephrine are a consequence of a constriction of the vasa vasorum. Such a condition led to a spasm of the aorta with the result that a local anemic state was set up leading to improper nourishment of the mural components of the blood vessel and, as a consequence, a degenerative state. It is of interest to note that Pearce and Baldauf reported typical medial arteriosclerosis in rabbits after single injections of epinephrine. Striking too, is the fact that the dose employed in this study was small. These workers report medial aortic damage in the rabbit 2½ months after the injection of 30 micrograms epinephrine (0.5 minims of a 1/1000 solution) in a 1 to 2 kg. rabbit. More

recently Friedman et al ('55) were able to produce a 50% incidence of this type of medial arteriosclerosis by repeated injections of epinephrine for a 15 day period. They also showed that pure epinephrine (100%) was approximately as potent as commercial epinephrine, which is usually contaminated with 10-25% arterenol. One hundred per cent epinephrine was approximately twice as potent as 100% arterenol.

b. Species Difference

Following Josue's initial experiment with epinephrine almost all of the work performed upon medial arteriosclerosis has been with the rabbit. Erb ('05) appears to be the first investigator to attempt to induce these lesions in animals other than the rabbit; i.e. the monkey.

Pearce and Stanton ('06) attempted to induce epinephrine lesions in young and old dogs. They report giving a 2.1 kg. puppy up to 1.3 mg. of epinephrine intravenously in repeated doses. Although respiratory and cardiac disturbances were observed in the dog, no evidence of arteriosclerosis was observed at autopsy. Unfortunately these workers do not indicate the length of their experiment or other details covering it.

Otto ('11) on the other hand extended his experiments on the dog for long periods of time and achieved success. Injections on four dogs lasted from 5 - 12 months. The doses of epinephrine employed were variable in that a dog weighing 5.5 kg. received 49.5 g. epinephrine in 78 injections during a five month period. Although the thoracic aorta was primarily involved, Otto also reported that large regressive medial foci were also present in the abdominal aorta. Less severe lesions were also sometimes

present in the innominate artery. Histologically there was fibrous thickening of the intima. Granular degenerative lesions were observed in the media of all animals. Occasionally the endothelium of the vasa vasorum showed endothelial thickening.

Beveri ('08) reported the production of atherosclerosis in the monkey by subcutaneous injections of epinephrine. The dose employed was 0.2 mg. progressively increased to 2.5 mg. epinephrine for the total animal. The arterial lesions induced by this procedure were supposedly identical to those encountered in the human. Unfortunately only one animal was used in this experiment indicating some question as to the validity of the conclusions reached by this worker.

Enger ('40) undertook a chronic epinephrine experiment on four dogs. One animal received from 0.2 to 50 mg. intravenous injections of epinephrine for 8 months while the other three dogs were thus maintained for about 2 years. Post mortem findings revealed left ventricular hypertrophy, hyaline deposits in the glomeruli and renal arterioles with occasional fat and amyloid changes. This study considered epinephrine as a factor in hypertension and probably because of this no mention was made of the aorta and larger arteries.

More recently Waters and de Suto - Nagy ('50) have been able to induce profound vascular lesions in dogs by intravenous administration of massive doses of epinephrine. Up to 4 mg. per day epinephrine was administered to dogs for a period of from 4 to 8 days. These massive doses were administered 1 mg./dog every 15 min. for a total of one hour. At autopsy segmental necrosis of many of the small coronary arteries was

observed. There also were extensive hemorrhages and medial necrosis of the pulmonary artery and aorta. Necrosis of the aortic adventitial vasa vasorum was evident as well as an occasional necrotic arterial lesion of the gastric mucosa. No evidence of renal or cerebral damage was observed.

The above are the only experimental animals employed in the production of epinephrine-arteriopathy. There is also an abundance of information suggesting that man is also prone to develop an arteriopathy as a consequence of the hyper-adrenalin state. Thus Eisenberg and Wallerstein ('32) in a study of 53 cases of pheochromocytoma report of an individual afflicted with the above malady and also having both hypertension and atherosclerosis. In the other cases reviewed about one-half of the individuals showed hypertension. Some of these hypertensive individuals also displayed atherosclerosis.

In 1933 Jorgensen presented a case report of hypertension probably due to ganglioneuroma. This individual showed arteriosclerotic lesions in the kidneys, brain and spinal cord.

Kremer ('36) reports that a young female, 14 years of age, afflicted with an adrenal medullary tumor, showed severe hypertension and arteriosclerosis of the vessels of the heart, lungs, brain, uterus, thyroid, ovaries and pancreas.

Kirschbaum and Malkin ('42) give an excellent case study of three individuals dying of pheochromocytoma. In all three cases the tumors were benign. Also, all of the individuals showed a pronounced hypertension. At autopsy, there was presence of severe arteriosclerosis in the coronary arteries, aorta and cerebral vessels. In each case there was cardiac

hypertrophy. In addition to these findings these clinicians chemically assayed the epinephrine content of the tumorous adrenal medulla. This revealed the presence of relatively large quantities of epinephrine. This study, in effect, presents a cause and effect relationship between high epinephrine titres within the human and the presence of arteriopathy.

Another case of pheochromocytoma reported by Tomaykowski et al. ('54) in a 54 year female revealed infarction and hyaline medial degeneration of the coronary arteries. The splenic artery and renal arterioles also showed medial involvement.

c. Inhibition

Since the first production of medial-epinephrine arteriopathy by Josue ('03) investigators were of the opinion that it was then possible to seek substances which would prevent or even reverse this arteriosclerosis. In fact this was one of Josue's primary goals in his investigations on arteriosclerosis with epinephrine.

It is not surprising therefore to note that as early as 1904 a claim was made for an experimental method of prevention of epinephrine arteriosclerosis. Lortat and Sabrouan ('04) performed thyroidectomies on a group of four rabbits. After a variable period following the thyroidectomy approximately 200 micrograms of epinephrine was injected in each rabbit on approximately alternate days for a period of a month or longer. At autopsy, the four rabbits treated in this way were reported as being totally free of lesions with the exception of one rabbit who displayed a dissecting aneurysm of the aorta. One rabbit was used as a control animal in this investigation and this animal upon epinephrine treatment displayed a

heavily sclerotic aorta. In addition, these workers report that the epinephrine sclerogenic regimen was comparable to that of Josue's ('03) in producing epinephrine lesions.

The results obtained by the above investigators were not confirmed by Loeb and Githens ('05). These investigators injected undefined quantities of epinephrine intravenously into a group of four thyroidectomized rabbits. The epinephrine administrations were presumably made on alternate days for a period of almost two months. Only one animal was observed to be free of lesions while the other three animals upon autopsy, displayed a slight to a severe degree of aortic damage, presumably epinephrine arteriosclerosis. This study appeared to be quite inadequate. No mention was made in the report regarding the state of the thyroid at the time of autopsy. Subsequently, no other reports regarding thyroidectomy and epinephrine-medial arteriopathy have been noted in the literature.

Various chemical agents have been employed in an endeavor to inhibit or prevent epinephrine arteriosclerotic lesions. The first experiment undertaken in this manner was that of von Koranyi ('06). Working on eleven rabbits, intravenous epinephrine was administered along with subcutaneous injections of 300 mg. doses of iodised sesame oil on approximately alternate days. After 8-21 such injections the animals were sacrificed and the aorta was inspected for plaques. His control group of 12 animals subjected to approximately the same treatment revealed at autopsy a 50% incidence of aortic plaque formation plus other vascular abnormalities. Of the eleven experimental animals used, 3 animals showed distinct plaques. From this experiment the author concluded that iodine (KI) exerts a pro-

fective influence on blood vessels exposed to an epinephrine sclerogenic influence.

Cummins and Stout ('06 - '07) also reported a protective effect initiated by potassium iodide upon epinephrine induced medial arteriosclerosis. Unfortunately only two rabbits and two controls were utilized in this study, two rabbits receiving epinephrine and potassium iodide while the other two received epinephrine alone. These workers failed to regress lesions once they were established by this method. Again only two animals were used in the study.

A more controlled investigation presented by Biland ('06) yielded results contrary to those reported above. Employing eight animals which received intravenous doses of epinephrine varying from 100 to 500 micrograms and concomitant administration of 0.1 to 1.5 grams of potassium iodide, Biland was able to protect only two out of the eight animals so treated. His controls consisted of 10 animals which received approximately the same amounts of epinephrine. From this group of animals, three did not show any vascular abnormalities. After close inspection of the vessels involved, Biland came to the conclusion that potassium iodide aggravates rather than protects against the sclerogenic action of epinephrine.

Nor did Loeb and Fleisher ('07) come to the conclusion that iodine preparations are protective. Utilizing 15 control rabbits - 11 out of 15 developed medial arteriosclerosis. Fifteen rabbits received the same epinephrine treatment as the controls plus addition of variable quantities of iodipin (iodized sesame oil). Of these, only two rabbits remained normal. Of twelve other rabbits receiving epinephrine plus

iodipin and/or potassium iodide, none was protected. These investigators came to the conclusion that it is not possible with the use of the above iodine preparation, to prevent arterial changes produced in rabbits by injection of adrenalin.

Strauss ('36), on the other hand, using large doses of potassium iodide together with colloidal silicic acid was able to prevent both experimental cholesterol atherosclerosis and also epinephrine arteriosclerosis. He reported a protective influence of 70% in the former group and only a 62% protective incidence in the latter group. This degree of protection was assumed to be significant by this investigator.

Haaper ('44) comes to the conclusion regarding the experiments performed with the thyroid or its products that, "the contradictory or unconfirmed nature of most of the claims made and the use of relatively small series of animals in the experiments do not permit any definite conclusions as to the actual effectiveness of the agents tried."

Other approaches utilized in inhibiting or preventing medial arteriosclerosis have been concerned with antagonizing the hypertensive properties of epinephrine. In this respect peripheral dilators and sympatholytic agents have most frequently been employed. Braun ('05) was the first to be cognizant of this possibility. The blood pressure antagonist employed was amyl nitrite. This prevented the hypertensive effects of epinephrine but did not prevent formation of medial lesions. He, therefore, postulated that medial arteriosclerosis is due to the toxic action of epinephrine and not simply due to a chronic rise in blood pressure.

Miller ('07) reports the exact opposite of the views presented above. Using amyl nitrite, nitroglycerin and sodium nitrate respectively this investigator was unable to permanently lower the hypertensive effects due to epinephrine. That is he reported only a transient lowering of blood pressure followed by a rise above normal. In addition, however, Miller reports that he was able to prevent epinephrine arteriosclerosis in four rabbits by this method.

Waters and de Sute - Nagy ('30) were able to prevent epinephrine arteriosclerosis in dogs by means of the adrenolytic, dibenamine administered intravenously and together with epinephrine.

In addition to the above mentioned substances, many other agents have been credited as being either partly or fully effective in the prevention or correction of epinephrine arteriosclerosis. Thus, Mansfield ('05) claimed success in this respect with choline. Lang & Szenthe ('30) reported that both insulin and ergotamine were able to prevent these lesions. Unfortunately, in all of these studies a rather small series of animals was utilized and the significance of these studies cannot be fully evaluated.

A few experiments have tried to correlate body fat and cholesterol changes in organisms receiving epinephrine. If one could show a hypercholesterolemia arising from chronic administration of epinephrine there naturally would be a basis for associating epinephrine arteriosclerosis with atherosclerosis. From such results the view could be entertained that both arteriopathies are actually quite similar and that, therefore, human arteriosclerosis is related to both experimental forms not simply -

atherosclerosis. Such an experiment has been performed by Kubo ('41). This investigator reported typical epinephrine arteriosclerotic lesions arising from injections of 20 - 100 micrograms of epinephrine from one to ten weeks. In addition this investigator reported that a hypercholesterolemia results from such a regimen. He also reports that potassium iodide prevented the aortic lesions but not the hypercholesterolemia.

These results did not conform to those by Bruger and Mesenthal ('34) who did not observe any significant changes in plasma cholesterol levels in five of six men following repeated injections of epinephrine. On the contrary, it has been reported by Alpern, Tutkewitch and Besuglow ('29) that an actual reduction in total and neutral blood lipids occur in dogs receiving injections of epinephrine. Dury and Moss ('54) in this connection, reported arteriosclerotic production in rabbits following repeated injections of epinephrine. They observed a small though significant fall in cholesterol-phospholipid ratios as well as a slight though significant rise in free cholesterol over total cholesterol ratio in the above treated animals. These findings appear to be incidental, apparently bear no relationship to the cholesterol atherosclerotic state.

4. The Thyroid Gland

Although thyroid gland and its secretory products are generally considered to be agents which prevent atherosclerosis, nevertheless, the hypersecretory state or excessive thyroid administration leads to the formation of cardiac as well as vascular abnormalities and arteriopathies. Menne et al. ('34) were able to induce myocardial lesions consisting of swelled muscle bundles, loss of cross striation, vacuolization and fibrosis

in 90% of rabbits made thyrotoxic with desiccated thyroid.

Heinlein & Dieckhoff ('36) observed severe damage of the medial components of medium-sized coronary arteries in cats experimentally made thyrotoxic. Doses varying from 0.20 mg./kg. to 0.75 mg./kg. thyroxine were injected intravenously daily or on alternate days. The lesions occurred after approximately one month treatment. These studies involved S. C. G. and heart - lung preparation changes though these were not very significant. Histological examination revealed a breakup of the elastic components and muscular degeneration of the coronary arteries. Fibrotic changes supervene over the affected areas. Degenerative myocardial changes resulted as a sequelae of these coronary lesions.

Von Balo ('39) observed severe degenerative changes within the aortae of rabbits. A dose of 0.5 mg. was injected subcutaneously into rabbits weighing approximately 2.5 kg. The aortic media was primarily affected. There was mucoid inhibition of medial components. This was followed by calcification. The intima was fibrous and proliferated. Regenerative elastosis was present above the medial lesions. Von Balo considered this type of lesion to be identical to that produced by epinephrine. This type of experimental lesion did not develop until approximately a month after the above type of treatment.

Mikulicich & Oester ('51) could not produce the above lesions by approximately the same thyroxine treatment in an experiment lasting approximately half the one month period described by Von Balo. Friedman ('55) injected smaller doses (0.15 mg./kg.) thyroxine subcutaneously for a period of two weeks to a group of 15 rabbits. Only one rabbit showed

any aortic changes. From this it may be concluded that adequate doses and a long term treatment is necessary for the development of thyroxine arteriosclerosis.

e. Combined Epinephrine-Thyroxine Medial Arteriopathy

The possibility of utilizing the sclerogenic potency of both epinephrine and thyroxine simultaneously have been explored by Mikulicich and Oester ('51). Utilizing large doses of epinephrine (50 micrograms/kg.) together with thyroxine administered subcutaneously (0.25 mg./kg.) 100% incidence as well as profound aortic vascular alterations were induced. The period of administration was reported as being only 26 days. The time involved in arteriosclerotic induction by the use of epinephrine alone is approximately one to two months. In consequence the use of the above method greatly facilitates the experimental production of medial arteriosclerosis. In addition the severe nature of the induced lesions could only be produced by epinephrine alone by long chronic epinephrine administration.

In 1952 Davis and Oester verified the above work and also attempted to inhibit epinephrine thyroxine arteriopathy by means of either ascorbic acid or inositol. Apparently massive doses appear to decrease the severity of this arteriosclerosis to a slight degree.

In a later work Davis ('54) employed various agents, usually thought to ameliorate or prevent the onset of atherosclerosis, in an attempt to prevent epinephrine-thyroxine induced arteriopathy. Some of these agents were also used in the prevention of a cholesterol arteriosclerosis induced by this investigator. Among the agents utilized are

adenosine triphosphate, adenosine monophosphate, progesterone, Heparin, and Alpha tocopherol. From this work it was concluded that adenosine triphosphate and Heparin significantly lower both the incidence and severity of the epinephrine-thyroxine induced lesions.

CHAPTER II

STATEMENT OF PROBLEM

this problem is concerned primarily with the action of the thyroid gland and its products upon epinephrine-induced-medial arterio-pathy. From the experiments of Mikulicich and Oester ('51) the fact has been established that exogenous thyroxin greatly augmented the sclerogenic potency of epinephrine but the degree of sclerogenesis, if any, induced by the thyroxin alone has as yet not been thoroughly investigated. Although there have been several reports describing the histological character of the vascular changes occurring as a result of chronic administration of epinephrine (Erb '05, Pearce and Stanton '06) nevertheless no reports are present describing the changes produced in vessels, other than the aorta, by means of epinephrine and thyroxine. Furthermore the reports concerned with the histological vascular changes occurring as a result of chronic epinephrine administration are conflicting. Because of these facts a survey of gross and histological changes occurring in several vital vessels appears to be in order. For this purpose three groups of animals, which are to be subjected to an epinephrine sclerogenic regimen plus variable quantities of thyroxine will be examined with a view toward histological changes occurring in:

1. The aorta

2. Cerebral arteries
3. Renal arteries
4. Pulmonary arteries
5. Coronary arteries
6. The femoral arteries

A large number of pharmacological agents have become associated with effects produced by the thyroid gland. A study of the following pharmacological agents in epinephrine arteriosclerosis and their effect thereon, be it, augmentary or preventative, would greatly enhance our knowledge concerning the nature of the sclerogenesis involved.

1. Iodide ion - Conflicting reports are present in the literature regarding the action of this substance in production of arteriopathy by epinephrine. As a consequence, a reinvestigation of this compound's action in medial arteriosclerogenesis appears to be of interest.

2. Dinitrophenol - 2,4 - Dinitrophenol is a compound which elevates the basal metabolic rate (Sollmann '48) as does thyroxine. This compound, however, has little else in common with this secretory product of the thyroid gland. As a consequence, the nature of thyroid sclerogenic activity might be better understood if an experiment were undertaken with concomitant administration of both epinephrine plus dinitrophenol. Augmentation of epinephrine sclerogenesis by dinitrophenol would indicate that a mere increase in metabolic rate appears to be accompanied by arteriosclerogenesis.

3. Thyretrophic Hormone - The thyretrophic hormone secreted by the anterior pituitary gland promotes endogenous secretion and synthesis of the actual utilisable products of the thyroid gland. A simultaneous administration of both epinephrine and thyretropin should therefore induce profound vascular changes if adequate amounts of endogenous thyroid substance are liberated and synthesized.

4. 3, 5, 3' - L-tri-iodothyronine (triiodothyronine) -

This compound has been recently discovered by Gross and Pitt-Rivers ('52). This compound appears to be four or five times as active as thyroxine itself (Gross and Pitt-Rivers '53). Furthermore this compound disappears much more quickly and has a shorter duration of action than does thyroxine (Blackburn et al. '54). This strongly suggests that this agent may be the active form of the thyroid hormone. If so, it would be of interest to realize the arteriosclerogenic potential of such a compound in the induction of epinephrine arteriosclerosis.

An investigation which would significantly help to document the role of the thyroid gland in the initiation of epinephrine medial arteriosclerosis, would be the administration of epinephrine to thyroidectomized animals. It appears logical, in a speculative sense, to accept the notion that if added thyroid substance greatly augments the sclerogenic activity of epinephrine, decreased amounts of, or abolition of thyroid activity will lessen the arteriosclerogenic potency of epinephrine. For this reason clarification of the issues

presented by Lortat and Sabarsanu ('04) and Loeb and Githens ('07) appear imperative for an understanding of this type of sclerogenic process. Therefore, a group of animals will be thyroidectomized and after adequate recovery will be subjected to epinephrine administration.

Effects similar to surgical thyroidectomy may also be initiated by the so-called antithyroid drugs. These compounds, thiourea, thiouracil, propylthiouracil and others, block the synthesis of thyroxine in the thyroid gland. More specifically, it appears that these compounds impair or prevent the iodination of tyrosine, a ready precursor of thyroxine (Astwood '49). This appears to be another feasible method of inducing a hypothyroid condition in an experimental animal. Such an animal subjected to repeated doses of epinephrine will then enable one to demonstrate the consequences of hypothyroidism in arteriosclerosis.

CHAPTER III

MATERIALS AND METHODS

In all of the following described experimental procedures the rabbit has been employed as the experimental animal. This animal was used for this study because it is readily prone to most types of experimental arteriopathies and because of other factors such as availability, ease of handling, etc. Sterile solutions and procedures were used in all parenteral administrations.

Method of Inducing Epinephrine Arteriopathy

All of the animals used in this study, with the exception of a small control group were subjected to the following epinephrine treatment. During the first day of treatment the animals received 25 micrograms/kg. of a 1/10,000 dilution of commercial epinephrine (Abbott; Parke, Davis). The following two days, this dose was increased to 40 micrograms/kg. On subsequent days the dose was 50 micrograms/kg. until the termination of this regimen. In all cases the intravenous route was used and in each experiment injections were made six times per week for a total of 15 injections. A group of ten animals received this treatment alone. This was considered to be a control group.

The Effect of Exogenous Thyroid Upon Epinephrine Arteriopathy

A group of 30 animals were subject to the above treatment plus three different doses of thyroxine. One group of 10 animals received epinephrine plus daily subcutaneous 0.05 mg./kg. amounts of D. L-Thyroxine (Roche-Organon).

A second group of 15 animals was subject to the same treatment except that these animals received 0.15 mg./kg. thyroxine. Five animals within this group died after only a few days of treatment and were excluded from this study. A third group of 5 animals were subjected to the treatment previously described with the exception that the dose of thyroxine was increased to 0.25 mg./kg.

At the termination of this procedure the animals were heparinized by the intravenous injection of 5,000 units of heparin (Abbott). Immediately following this, the animals were sacrificed by over-dosage of thiopental. The thoracic cavity was opened and the pulmonary artery was cleared, excised, washed in saline and fixed in Zenker's solution. A portion of the hilus of the lung was also fixed in the same manner. Following this, the thoracic aorta and heart were separated from the dorsal body wall and adjacent tissue, in toto. The aorta and coronary arteries were then perfused with physiological saline, followed by Zenker's solution. The aorta was then slit longitudinally and grossly graded as to presence of sclerosis. This entire portion of tissue was then fixed in Zenker's solution. A portion of kidney and femoral artery were treated in the same manner. The dorsal portion of the skull

was then opened and removed. A portion of the brain was then excised. This extended from the medulla to the optic chiasma. Care was taken not to damage unduly the vessels at the base of the brain. This specimen was also washed in saline and after careful trimming, fixed in Zenker's.

Fixation lasted 24 hours after which the tissue was washed for 24 hours. It was then dehydrated in ethyl cellosolve, cleared in benzene, infiltrated in several baths of paraffin and embedded. Sections were cut at 5 to 10 micra. Four different sections were taken from the heart extending equally from the apex to the base of the heart. The slides containing the sections were treated in the usual manner with the exception that cellosolve was used as the dehydrating agent. These were then stained with hematoxylin and eosin.

A few aortas were grossly stained with Sudan IV. In a group of five animals frozen sections were taken of the heart, stained with Sudan IV and counterstained with hematoxylin.

A group of 10 animals were treated in the same manner as were the epinephrine animals with the exception that physiological saline instead of thyroxine was administered intravenously. These animals received comparable volumes of saline and they also were treated for a 15 injection period. Histological examination of these animals was identical to that described above.

Gross gradation of aortic lesions was made by subjective observation. Though the method was subject to error it was nevertheless

adequate for an estimation of the severity of large vascular lesions.

Gradation was carried on in the following manner in all experiments: 0 (Zero)

- normal or non-pathologic aorta, 1 - small, single elevations or plaques, 2 - multiple light plaques, 3 - multiple to more numerous severe plaques, 4 - confluent severe plaques engulfing a large portion of the vessel. The following studies were graded on the above basis as were those previously mentioned.

2.4 - Dinitrophenol and Epinephrine Arteriopathy

Dinitrophenol (Matheson, Coleman, and Bell) was administered to a group of 15 animals, subcutaneously, with simultaneous administration of the usual epinephrine sclerogenic regimen. A 2% dinitrophenol solution was used, dissolved in a 2% NaHCO_3 solution. This was Zeitz filtered and kept in sterile container. The dose of dinitrophenol employed varied from 30 to 75 mg./kg. per day. One half this dose was administered at the time of epinephrine administration and the other half was administered two hours later. The larger doses of dinitrophenol were quite toxic and as a consequence, a second group of ten animals was given 35 mg./kg. of dinitrophenol and epinephrine.

Potassium Iodide (KI) and Epinephrine Arteriopathy

A group of ten animals received 1.0 g KI (Merek, U.S.P.)/kg./day plus the already mentioned amounts of epinephrine. A 20% aqueous solution of KI was used. The solution was administered orally by stomach tube. This dose of KI appeared to be too toxic and as a result a second group of animals were given 0.5 gm. KI/kg. body wt. three times each week.

Thyrotropin (T.S.H.) and Epinephrine Arteriopathy

A group of ten animals received 0.05 unit of T.S.H., I.V. (Thyrotropar - Armour & Co.) per kg. body weight. Two hours following this administration intravenous epinephrine was administered in the manner previously described. This dose may have been too small although Reichlin and Reid ('55) reported maximal effects with such an intravenous dose. At any rate, a second group of 10 animals was used with twice the amount of T.S.H. employed.

Although T.S.H. is not "official" there nevertheless is a U.S.P. reference standard. A unit of potency, according to this standard, is defined as the thyrotropic activity of .20 mg. of the U.S.P. reference substance. All of the above doses are expressed according to this standard.

1, 5, 3' - L-Tri-iodothyronine and Epinephrine Arteriopathy

According to previous reports triiodothyronine sodium (Smith, Kline, and French) appears to be approximately 6-9 times as potent as the sodium salt of D, L-thyroxine (Gross and Pitt-Rivers '52). A group of ten animals were therefore given 5 micrograms per kg. subcutaneously plus the usual epinephrine sclerogenic regimen. After 5 injections the dose was increased to 10 micrograms per kg. and after 10 injections the dose of triiodothyronine was increased further to 20 micrograms/kg. A second group of ten animals were used and these were given 50 micrograms/kg. subcutaneously plus the usual epinephrine amounts.

Thyroidectomy and Epinephrine Arteriopathy

A group of 13 animals was surgically thyroidectomized under sodium pentobarbital anesthesia. An intravenous dose of approximately 35 mg./kg. was used. In order to facilitate handling and because of the fact that cutaneous perception was appreciable the entire neck area of the rabbit was infiltrated with 2% procaine. After exposing the trachea, as complete a bilateral thyroidectomy as possible was performed. Following the operation, each animal received 100,000 units of penicillin. Seven days was allowed for recovery. After this period each animal was subject to the usual epinephrine sclerogenic regimen.

In an endeavor to determine how completely the thyroidectomy had been accomplished, 5 microcuries of I^{131} (Abbott) was administered subcutaneously to each of ten of the above animals, 24 hours before being sacrificed. The trachea and adjacent tissue areas, where thyroid tissue is usually present, was then removed. In addition, an indifferent muscle area of the thigh was removed and considered a control area. The thyroid area and thigh muscle were then separately digested in 10% NaOH. The resulting solutions were counted by a standard Geiger-Müller counter and corrected for volume. A group of 5 untreated animals were similarly injected with radio-iodine and examined in the same manner.

Propylthiouracil (6-n-propyl-2-thiouracil) and Epinephrine Arteriopathy

Ten animals received daily oral (stomach tube) administrations of

125 mg./kg. amounts of propylthiouracil (Delta) suspension. A 5% suspension was made in a basic medium ($1\% \text{NaHCO}_3$) and stirred mechanically. The epinephrine sclerogenic regimen was instituted after 5 administrations of propylthiouracil. The propylthiouracil administration was continued along with the epinephrine until the termination of the experiment. Epinephrine injections were made approximately 2 hours after the administration of propylthiouracil.

In order to determine the effects of the propylthiouracil upon the state of the thyroid gland, a portion of this gland was removed at autopsy and sectioned.

The above procedures are briefly summarized in Table I.

TABLE IOUTLINE OF PROCEDURES

Regimen	Number of Animals	Dose (mg./kg.)	Route
Epinephrine	15	0.05	IV
I. Epinephrine plus Thyroxine	10	0.05 0.05	IV Sq
II. Epinephrine plus Thyroxine	15	0.05 0.15	IV Sq
III. Epinephrine plus Thyroxine	5	0.05 0.25	IV Sq
Epinephrine plus diatrizoate	25	0.05 a. 60 b. 35	IV Sq Sq
Epinephrine plus T.S.H.	20	0.05 a. 1(0.05 unit) b. 2(0.1 unit)	IV IV IV
Epinephrine plus KI	20	0.05 a. 1000 b. 500 (3 times/wk.)	IV Oral Oral
Epinephrine plus Triiodothyronine	20	0.05 a. 0.005, 0.010, 0.020 b. 0.050	IV Sq Sq
Epinephrine plus propylthiouracil	10	0.05 125	IV Oral
Epinephrine plus Thyroidectomy	15	0.05	IV

CHAPTER IV

RESULTS

A. Gross & Histological Vessel Survey of Epinephrine and/or Thyroxine Treated Animals

1. The Aorta

Careful examination of the blood vessels revealed greatest arteriopathic involvement within the aorta. The media is primarily involved. Initially there is a separation of the medial elastic and muscular components. Minimum effects were observed in animals treated with epinephrine alone. (Figure 2).

The aortae of animals receiving epinephrine plus exogenous thyroxine generally revealed a more severe type of arteriopathy. Gross examination revealed a generalized thickening of the entire wall. In addition, calcification was a prominent characteristic. Calcified areas were usually located within regions that showed separation. This part of the aorta was thinner or more compressed than the adjacent more normal vicinities. Other areas showed a marked increase of calcareous material so that a mass of the calcified material could be observed. Usually, the most numerous and the most severe lesions were located around the arch of the aorta and near the semilunar valves. The incidence of lesions

decreased as one progressed caudally along the thoracic aorta. In general, only the thoracic aorta was involved. Only an occasional animal showed lesions in the abdominal aorta and these were at a point near the iliac bifurcation. Such gross lesions are shown in Figure 3.

Histologically the sclerotic aortas showed marked degenerative and necrotic changes of the medial components. The elastic and muscular components appeared to be separated, fragmented and largely replaced by calcium. The calcareous deposition was located in the central portion of the media and gave the appearance of being a long homogenous shaft. Some areas become fragmented. This fragmentation may be real or may be an artifact of the technique employed. Hyaline degeneration also appears to be prominent and was mostly present in areas surrounding or adjacent to the calcified tissue (Figure 4).

Proliferative changes of the intima were apparent in most animals treated with epinephrine and thyroxine. However the amount of proliferation was rather minimal varying only a few layers in thickness from the normal. This proliferation of the intima appears as a compact band of closely packed endothelial cells just above the internal elastic membrane. (Figure 5). That these cells are not atheromatous or lipid laden is evidenced by the fact that they will not selectively pick up Sudan IV. The intinally proliferated areas show no presence of foam cells which is also characteristic of atheromatous arteries.

2. The Pulmonary Artery

The pulmonary arteries of rabbits treated with epinephrine and thyroxine appeared to be thickened when observed grossly. In spite of this no gross plaques were apparent. Histological examination showed diffuse degenerative changes. This type of arteriopathy was mainly in the media. The muscular as well as the elastic components undergo degenerative changes. The central portion of the media of the pulmonary artery is affected. This area becomes devoid of nuclei and in general has poor staining characteristics. The area became hyalinized (Figure 7). The hyalinization is usually associated with a thickening of the entire wall of the artery although some arteries show thickened areas without hyalinization. Smaller arteries within the hilus of the lung are similarly affected although to a lesser degree. Only relatively small patches of degenerated tissue were apparent in these areas (Figure 9). None of the pulmonary arteries or its tributaries displayed any areas of calcification. As mentioned, hyalinization was the primary change observed.

3. Other Vessels -

The cerebral, coronary, and renal arteries did not show any marked changes (Figure 11, 13). Sections of the heart stained with hematoxylin and eosin revealed coronaries which appeared to have a scarcity of elastic fibers. However, with orcein, a stain for elastic fibers, staining indicated that the elastic fibers of the coronaries were intact, the internal elastic membrane was complete and entire. (Figure 14).

A few of the femoral vessels did display degenerative changes. These vessels showed medial degeneration and hyalinization changes (Figure 16). Apparently large trunks are the vessels primarily affected, small arteries and arterioles did not appear to be altered.

No vascular changes were observed in ten control animals treated with intravenous injections of sterile saline.

3. The Effect of Varying Thyroid Treatment Upon Epinephrine Arteriosclerosis

1. Epinephrine Arteriopathy and Thyroxine

Rabbits treated intravenously with a 15 injection regimen of epinephrine showed a 50% incidence of arteriopathy. The lesions which were induced were mild, and at the most only of a second degree severity. This is in general agreement with the observations of Friedman et al (1955). When exogenous thyroxine was added to the epinephrine both the incidence and severity of the lesions were increased. The animals receiving 0.05 mg./kg. thyroxine subcutaneously plus daily injections of epinephrine displayed an 80% incidence of aortic lesions after 15 days of treatment. The average degree of sclerosis, which is the sum of the individual measurements of sclerosis in a particular group of animals divided by the number of animals within this group, was 2.2. When the dose of thyroxine was increased to 0.15 mg./kg. and 0.25 respectively in two other groups of animals a 90% and 80% incidence of arteriosclerosis occurred respectively. The average degree of

sclerosis was increased to 3.0 and 2.4 respectively in these two groups of animals. These results are summarized in Table II.

TABLE II

PRODUCTION OF MEDIAL ARTERIOPATHY WITH EPINEPHRINE AND EPINEPHRINE PLUS
THYROXINE

Treatment	# of Rabbits	Deaths Prior to 6 days	Death 6-15 Days	Animals Surviving Experiments	Degree of Sclerosis 0-1-2-3-4	% Incidence	Average Degree of Sclerosis
E	10		1	9	5 4 1 0 0	50	0.6
ET ₁	10		2	8	2 1 2 3 2	80	2.2
ET ₂	15	5	2	8	1 1 1 3 4	90	2.8
ET ₃	5		4	1	1 0 1 2 1	80	2.4

* E - Commercial Epinephrine HCl; T-Thyroxine (T₁ - 0.05 mg./kg.; T₂ - 0.15 mg./kg; T₃ - 0.25 mg./kg.

In series ET₂ the animals dying prior to seven days of treatment though included in the table for the sake of completion are excluded from the actual percentage data.

2. Epinephrine Arteriopathy and Triiodothyronine

Animals receiving triiodothyronine in amounts ranging from 0.005 - 0.020 mg./kg. did not reveal a greater tendency toward the development of medial arteriosclerosis than did animals treated solely with epinephrine. An incidence of sclerosis of 33% was observed. The average degree of sclerosis was 0.66. When the dose of triiodothyronine was increased to 0.05 mg./kg. an 80% incidence of arteriopathy was observed with an average

degree of sclerosis of 2.5. These results are summarized in Table III.

TABLE III

PRODUCTION OF EPINEPHRINE - TRIIODOTHYRONINE ARTERIOPATHY

Treatment	# of Rabbits	Deaths Prior to 6 Days	Death 6-15 Days	Animals Surviving Experiments	Degree of Sclerosis 0-1-2-3-4	% Incidence	Average Degree of Sclerosis
N	10		1	9	5 4 1 0 0	50	0.6
2Tr ₁	10	1	4	5	6 2 1 0 0	33	.4
2Tr ₂	10	0	5	5	2 1 0 4 3	80	2.5

*Tr₁ - Triiodothyronine - 0.005 - 0.020 mg./kg. Tr₂ - Triiodothyronine 0.05 mg./kg.

The type of lesion induced is mainly in the media and appears to be the same type as that induced by epinephrine, or that which is seen when combined epinephrine - thyroxine treatment is undertaken. This was verified by histological examination of the sclerosed aortas of these animals. (Figure 17).

3. Epinephrine Arteriopathy and Thyrotropin (T.S.H.)

Two groups of animals were utilized in this experiment. One group of animals received 0.05 units thyrotropin/kg. daily by the intravenous route, plus epinephrine, while the second group received 0.10 units of thyrotropin. This agent appeared to be rather toxic at the outset of the experiment and several animals died early in treatment. The result of these two experiments are tabulated in Table IV. No real

change in incidence of sclerosis between this group and the epinephrine group was observed as a result of this treatment.

TABLE IV

THE EFFECT OF THYROTROPIN ON EPINEPHRINE ARTERIOPATHY

Treatment	# of Rabbits	Deaths Prior to 6 days	Death 6-15 Days	Animals Surviving Experiment	Degree of Sclerosis 0-1-2-3-4	% Incidence	Average Degree of Sclerosis
E	10		1	9	5 4 1 0 0	50	0.6
E Th ₁	10	0	0	10	6 2 2 0 0	40	0.6
E Th ₂	10	2	2	6	5 2 1 0 0	37	0.5

* Th₁ - 0.05 unit T.S.H./kg. I.V.:

Th₂ - 0.1 unit T.S.H./kg. I.V.

Examination of the aortic lesions revealed that the arteriopathy induced was not unlike that observed with epinephrine alone (Figure 18).

4. Epinephrine Arteriopathy and Dinitrophenol (D.N.P.)

Two groups of animals were utilized in this experiment. Initially one group of 15 animals received varied doses of dinitrophenol (30 - 75 mg./kg. S. Q.) while the second group received a stable dose of 40 mg./kg. dinitrophenol. Of course, all animals were subject to the same epinephrine treatment. Dinitrophenol did not seem to lower or increase the incidence of epinephrine arteriopathy as shown in Table V.

TABLE VTHE EFFECT OF DINITROPHENOL UPON EPINEPHRINE ARTERIOPATHY

Treatment	# of Rabbits	Deaths Prior to 6 Days	Death 6-15 Days	Animals Surviving Experiment	Degree of Sclerosis 0-1-2-3-4	% Incidence	Average Degree of Sclerosis
E	10		1	9	5 4 1 0 0	50	0.6
E (D.N.P.) ₁	15	7	0	8	5 2 1 0 0	37	0.5
E (D.N.P.) ₂	10	0	3	7	6 2 2 0 0	40	0.6

* (D.N.P.)₁ - Variable quantities of D.N.P.

(D.N.P.)₂ - 40 mg./kg. D.N.P. administered subcutaneously.

The type of arteriopathy observed to occur with dinitrophenol plus epinephrine appears to be medial and for all practical purposes the same as that observed with epinephrine alone (Figure 19).

5. Epinephrine Arteriopathy and Potassium Iodide (KI)

Two groups of animals were used in this study. The first group of ten rabbits received 1 gm. KI/kg./day plus epinephrine. This dose was much too toxic and all of the animals died before the termination of seven days treatment. None of the animals demonstrated any gross aortic lesions. A second group of 10 animals were given 0.5 gm. KI/kg. three times per week plus the usual epinephrine treatment. Such a regimen was not without toxic effects, all of the animals survived beyond six days and three had gross aortic lesions, though extremely mild. Histological examination of these aortae revealed a small amount of medial

damage in two of the animals. No abnormalities were found in the other animal but the possibility still exists that slight medial damage may have been present. The type of medial lesion apparent is shown in Figure 20. The results of the experiment are summarized in Table VI.

TABLE VI

THE EFFECT OF POTASSIUM IODIDE UPON EPINEPHRINE ARTERIOPATHY

Treatment	# of Rabbits	Deaths Prior to 6 Days	Death 6-15 Days	Animals Surviving Experiment	Degree of Sclerosis 0-1-2-3-4	% Incidence	Average Degree of Sclerosis
N	10		1	9	5-4 1 0 0	50	0.6
N KI ₁	10	10					
N KI ₂	10	0	6	4	0 3 0 0 0	30	0.3

* KI₁ - 1 gm./kg./day.

KI₂ - 0.5 gm./kg./3 times/wk.

C. Agents and Procedures Utilized which Lessen or Inhibit Epinephrine Arteriopathy.

1. Thyroidectomized Animals Receiving an Epinephrine Sclerogenic Regimen.

Thirteen animals were successfully thyroidectomized. After adequate recovery these animals received a sclerogenic regimen of epinephrine. At autopsy no gross aortic lesions were observed nor did histologic examination of each of their aortas reveal any lesions. The aorta observed in

Figure 21 is that of an epinephrine treated thyroidectomized animal and is essentially identical to a normal aorta.

At the termination of the above experiment radioiodine was injected into the animals. After an interval of 24 hours the area surrounding the site of the thyroid gland was digested and counted in liquid state for radioactivity. The same was done for an indifferent area of muscle from the thigh. A group of 5 normal non-thyroidectomized animals were also subjected to the same procedure. The concentration of radioactive iodine in the counted areas should yield some indication of iodine concentrating activity in the areas in question. In the thyroid area these measurements therefore yield information regarding the completeness of the thyroidectomy. Results of these procedures are indicated in Table VII. All counts are corrected for background and volume.

TABLE VII

¹³¹I UPTAKE IN THE NECK REGION OF NORMAL AND THYROIDECTOMIZED RABBITS

Thyroidectomized Animal #	Thyroid Area C/M	Thigh Area C/M	Normal Animal #	Thyroid Area C/M	Thigh Area C/M
7	12,899	16	1	65,290	117
10	672	78	2	29,518	92
13	30	13	3	42,321	76
14	6,166	--	4	24,567	85
20	3,543	35	5	56,390	262
23	7,690	25		43,617	126
24	2,861	203			
25	3,165	46			
26	3,470	57			
30	990	52			
Average -	4,419	52			

Thus, if the normal rabbits are considered as containing 100% of their normal thyroid tissue, revealed by the above procedure, then the thyroidectomized animals possessed approximately 10% of the ability to concentrate iodine as compared to those possessing normal thyroid. Perhaps this indicates that the thyroidectomy was not complete, due either to incomplete surgical extirpation or to restoration of thyroid activity in the residual areas. It does however indicate that a markedly hypothyroid animal was available for this study.

2. Epinephrine Arteriopathy and Propylthiouracil.

A group of ten animals received epinephrine plus 125 mg./kg. propylthiouracil daily by the oral route. At the termination of the experiment the aortae of these animals were examined. All of the animals displayed gross lesions. The lesions were not severe being approximately of a second degree. Histological examination of these vessels revealed that the arteriopathy, though of a medial type was present in tissue components just adjacent to the intima and sometimes encroaching upon the intima itself. (Figure 22). Examination of thyroid glands of these animals revealed a normal state. The epithelium varied from cuboidal to a low cuboidal state. The colloid present was not an unduly large amount for normal rabbit thyroid. All of this indicates that the glands were in a relatively normal state and had not been affected by the propylthiouracil (Figure 24).

The above high incidence of sclerosis indicated that the

propylthiouracil did not act as an inhibitor of arteriosclerosis, as might have been anticipated. A second group of 10 animals was treated with propylthiouracil without epinephrine. These animals received 125 mg./kg. propylthiouracil by stomach tube, daily, as a 5% suspension for a period of 15 administrations. Following this treatment period the animals were autopsied. The results of this experiment and the previous one are tabulated in Table VIII.

TABLE VIII

EPINEPHRINE - PROPYLTHIOURACIL AND PROPHTHIOURACIL ARTERIOPATHIES

* Treatment	# of Rabbits	Deaths Prior to 6 Days	Death 6-15 Days	Animals Surviving Experiment	Degree of Sclerosis	% Incidence	Average Degree of Sclerosis
					0 1 2 3 4		
E	10		1	9	5 4 1 0 0	50	0.6
EP	10	0	0	10	0 1 8 1 0	100	2.0
P	10	2	1	7	4 1 3 1 0	56	1.1

P - 125 mg. propylthiouracil/day.

As indicated in the table 56% of the animals exhibited aortic lesions. This same group had an average sclerosis of 1.0. Both the aortas and the thyroid glands of the sclerosed animals were examined histologically. In order to better observe the distribution of the elastic fibers the aortas were stained with iron hemotoxylin. Some of the aortas were also stained with orcein as well as with the usual

hematoxylin eosin procedures (Figures 26 and 30).

Iron hematoxylin staining of the thyroid gland showed that the colloid material was a grayish color which is characteristic of the normal state. It could be concluded that this was normal stored colloid. Some vacuolization of colloid appeared which might be related to propylthiouracil activity but possibly may be an artifact. (Figure 28). The epithelium was of the cuboidal type and this also indicated that the gland was in a functionally normal state.

CHAPTER V

BICOMETRIC ANALYSIS

Although the results accumulated in the above outlined experiments appeared to yield concise definite results they were nevertheless subject to statistical analysis. All of the experiments were compared to the epinephrine group of animals which will be considered to be the standard or the basis for comparison for all of the experiments performed. That there is a real difference between the incidence and number of lesions in the epinephrine group of animals (E) and the saline control group of animals displaying no lesions is obvious. Comparing these two groups statistically by chi-square (X^2) analysis reveals a significant difference with a probability of less than 0.05.

Utilizing the Null Hypothesis as a method of analysis, the degree of sclerosis in the experimental group of animals was compared to the sclerosis in the epinephrine group as indicated by Table IX.

(TABLE IX - SEE FOLLOWING PAGE)

As indicated, there is a real and not a chance difference between the following three groups, epinephrine thyroxine animals (ET), epinephrine-triiodothyronine animals (ET₂), epinephrine-propylthiouracil animals and the standard or control epinephrine animals.

TABLE IX

STATISTICAL EVALUATION OF DATA WITH REFERENCE TO INCIDENCE AND DEGREE OF
MACROSCOPIC SCLEROSIS

Treatment	# Animals	Degrees Freedom	Mean Sclerosis	T	Probability (Compared to Group C)
NT ₁	10	9	2.2	3.10	0.01
NT ₂	10	9	2.8	4.45	0.01
NT ₃	5	4	2.4	3.12	0.01
NT ₁	9	8	0.4	0.49	0.5
NT ₂	10	9	2.5	3.48	0.01
ETH ₁	10	9	0.6		Very large
ETH ₂	8	7	0.5	0.28	0.5
E (DNP) ₁	8	7	0.5	0.28	0.5
E (DNP) ₂	10	9	0.6		Very large
E KI ₂	10	9	0.3	1.11	0.2
E P	10	9	2.0	5.24	0.01
E	10	9	0.6	-	-

In another test for significance all of the epinephrine - thyroxine animals were placed in a single group and compared to the epinephrine standard group. In addition the thyroidectomized group of animals (OT) was also compared to the epinephrine control group. The Chi-square (χ^2) method of analysis was used to compare these groups on the basis of incidence of aortic lesions. These results are summarized in Table X.

TABLE X

STATISTICAL EVALUATION OF DATA WITH REFERENCE TO % INCIDENCE OF
MACROSCOPIC SCLEROSIS

<u>Treatment</u>	<u># Animals</u>	<u>χ^2</u>	<u>P.</u>
ET	35	1.0	not significant
OT	13	5.4	0.05
E	10	-	-

CHAPTER VI

DISCUSSION

These studies were undertaken with the expressed purpose of clarifying the functional role of the thyroid gland in the production of epinephrine arteriopathy. To this end, a final solution of the issues concerned with arteriosclerogenesis was not attempted, but from the data and observations procured one might speculate in terms of the broader problems concerned with arteriosclerogenesis. For this purpose, a reconsideration of the general theories of arteriosclerogenesis in the light of new findings presented in this dissertation appears to be in order.

1. The Senescence Theory -

Little evidence could be derived from this study regarding the validity of the "aging" theory. This situation prevails because of the fact that the very essence of the senescence theory is based upon human clinical observations. The observations in support of this concept are based on pathologic and other arterial changes which are claimed to be apparently associated with aging. Any experimental studies undertaken to support these views are automatically stifled in that a lifetime or more would probably be needed for adequate and accurate appraisal.

It is of interest to note, however, that the aortic lesions observed in this investigation were often extremely calcified and necrotic. Necrosis and calcification of blood vessels are considered to be the main characteristics of aging by believers of the senescence theory. Yet, in this study, such extreme "age changes" were observed in a matter of approximately two and one-half weeks.

Of greater importance is the logical conclusion which would ensue if the senescence theory were accepted as fact. It might be concluded that all research regarding arteriosclerosis should be halted since aging is inevitable. Such a fatalistic attitude should be discarded.

2. Relation to Mechanical Trauma Theory

Although no conclusion could be reached regarding the validity of the mechanical trauma theory on the basis of this investigation, the tenets of this theory are by no means excluded. Undue stretching of arterial, especially aortic, mural components as a result of a greatly increased blood pressure and especially a large pulse pressure may result in medial injury. This injury might induce compensatory or degenerative changes which would alter the integrity of the arterial wall. Such a proposition has been proposed by Thoma (1886). It is indicated in the results of this investigation and those of Mikulicich and Oster ('51) that thyroxine augments atherogenesis. It is also conceivable that epinephrine blood pressure effects might be greatly increased by added thyroid substance especially in a study such as

undertaken here. Pulse wave contours might also be affected with repeated administrations of epinephrine and thyroxine. Such an investigation including blood pressure and pulse pressure recordings would appear to be very basic to a proper understanding of arteriosclerogenesis.

3. The Toxin Theory

The view that certain substances of bacterial origin are of consequence in the induction of arterial lesions has not received much support from investigators in the field. Some human arterial lesions such as syphilitic aortitis are due to bacterial invasion but there is little evidence that the usual human arteriopathies are of bacterial origin as proposed by Klotz ('06). Brann ('05) was of the opinion that epinephrine arteriopathy might be due to a direct toxic reaction of epinephrine upon aortic medial components. This notion is hardly tenable in that epinephrine arteriopathy appears to be inhibited in the presence of sympatholytic substances although this appears to be controversial. The present study does not support the possibility of a direct aortic cytotoxic effect for epinephrine in that epinephrine arteriopathy could not be produced in thyroidectomized animals. Because of the fact that other agents, such as cholesterol, produce an arteriopathy closely resembling human arteriosclerosis, the toxin theory of arteriosclerogenesis has not received widespread support.

4. The Hypoxia Theory

Of all of the theories proposed to explain arteriosclerogenesis the Hypoxia theory appears to be most widely accepted. This concept

considers arteriosclerogenesis to be a reaction of arteries to the hypoxic state. One of the chief reasons this theory maintains widespread acceptance is because of its comprehensibility, since this might conceivably be a primary factor in the induction of most, if not all, of the known arteriopathies.

Little information regarding the factual establishment of the hypoxia theory can be derived from this study. Hypermetabolic states as produced by thyroxine appear to augment epinephrine sclerogenesis. However, such a general effect could hardly be considered to establish the validity of the hypoxia theory. In order to better determine what influence, if any, the hypoxic state has upon arteriosclerogenesis a study of the cellular metabolic state at the site of arterial lesions would have to be undertaken. The literature reveals very few cellular studies of this type.

In view of the proposed theories of arteriosclerogenesis and their relationship to this study, a consideration of the primary results of this dissertation will not be presented.

I. The Role of the Thyroid in Epinephrine Arteriopathy - Anatomical Considerations

Histology

The histological results observed in this study are in accord with the studies of Erb ('05), Scheidemann ('05) and other work concerned with epinephrine arteriopathy. The addition of thyroxine definitely

augments the sclerogenic effect of epinephrine (Mikalieich and Cester '51) in that the severity of the lesions is greatly augmented. The aorta was the vessel primarily affected under epinephrine-thyroxine treatment. Other vessels such as the pulmonary and femoral arteries were influenced to a lesser degree. It is of interest to note that calcification changes were primarily associated with the aorta while hyalinization degenerative changes were associated with the pulmonary and femoral vessels. It is possible that the hyalin changes represent early pre-calcification areas.

It appears to be clearly evident that epinephrine-thyroxine arteriopathy in the manner in which it was produced here, is associated with larger vessels. The coronary, renal and cerebral arteries were not affected. The fact that some investigators (Klotz '06) and others have compared this arteriopathy with that described by Moenckeberg ('03), or Moenckeberg's arteriosclerosis, is misleading in that Moenckeberg's arteriosclerosis is most often observed peripherally. The experiments of Waters and de Suto-Nagy ('30) upon the dog appear to be qualitatively different in this respect in that these investigators observed degenerative changes in small arteries and arterioles. It should be indicated, however, that the procedures utilized by these investigators were different from those employed in this study. These investigators administered epinephrine doses which were approximately ten times as great as those employed in this study. Administrations were also more frequent. It is reasonable to conclude that a somewhat different response could be elicited utilizing

such procedures.

Most of the degenerative changes evident from epinephrine and/or thyroid treatment were observed invariably in the media. It apparently does not matter whether the affected artery is elastic or primarily muscular. Most investigators (Erb '05, Klotz '06, etc.) have stressed the notion first proposed by Josse ('03) that the elastic connective tissue areas of the media are initially affected. Our studies indicate that even though the greatest involvement is in the largest arteries, medium sized arteries may also be affected as exemplified by the hyalinization changes observed in the pulmonary and femoral vessels.

Intimal proliferation is rather common in the epinephrine or combined epinephrine-thyroxine arteriopathy. This is not meant to indicate that the arteriopathy studied here is identical with atherosclerosis or human arteriosclerosis in which intimal changes also occur. Dissimilarity is evident by the fact that no lipid infiltration of the intima characteristic of human atherosclerosis was observed. Further the intimal proliferative changes observed in this study consisted of a compact endothelial stratification. No indication of foam cell accumulation was observed. However, it should be pointed out that medial changes are also characteristic of human arteriosclerosis and perhaps on this ground there may be a basis for comparison of the two arteriopathies. Certainly, the intimal changes in the epinephrine-thyroxine aortae usually developed superimposed over the altered media. This lends some support to the notion of Duff ('35) and Hirsch and Weinhouse ('43) who contend that

the medial changes precede the development of atheromata.

II. The Effect of Alterations in Thyroid Activity Upon Epinephrine Sclerogenesis.

Thyroid Sclerogenic Action

The epinephrine - thyroxine sclerosis study presented here has clearly demonstrated that normal thyroid secretory activity is essential for the induction of this type of medial arteriopathy. It can be definitely stated that exogenous thyroid substance whether it be in the form of thyroxine or triiodothyronine, augments the sclerogenic potency of epinephrine. That thyroxine is a sclerogenic substance in its own right has been postulated by Heinlein and Dieckhoff ('36) and von Balo ('39). Their results were not verified by Mikulicich and Oester ('51) or Friedman ('55). The reason that these last two groups of workers failed to obtain similar results was possibly due to the fact that the latter experiments were of shorter duration and less thyroxine was administered.

Of more significance is the fact that this type of experimental medial arteriopathy is not induced in animals experimentally made markedly deficient in thyroid. Such results serve to clarify the observations of Lortat and Sabereanu ('04). Such results also lead to the postulate that it is the thyroid gland and not the sympathomimetic effects of epinephrine which are the actual causative inducing factors in this type of medial arteriopathy. In order to completely verify such an hypothesis, animals deprived of endogenous adrenergic substances would have to be subjected to

thyroxine treatment. Such an experiment is not feasible. One is left with the conclusion that both thyroxine (thyroid secretion) and epinephrine (adrenergic secretion) have mutual augmentary sclerogenic activity.

The results obtained using thyrotropic hormone in general do not conform with the above postulate. Epinephrine sclerosals should have been augmented due to increased thyroid activity induced by the anterior pituitary thyrotropic hormone. It should be pointed out that the doses of thyrotropin employed in this study approach lethal levels as indicated in a study by Reichlin & Reid ('57) for the rabbit. Lethal effects were also observed in this study in that two early deaths were observed when high dose levels of thyrotropin were employed. The deaths may be a result of anaphylactic responses due to the protein hormone. From these results a number of possibilities might account for the responses observed in this study:

1. Ineffective Thyrotropin (TSH) - the thyrotropin available for this study was considered the best available commercial preparation. (Thyrotropar - Armour and Co.). The possibility that the compound was impotent is remote because of the fact that it was assayed shortly before use in this study.

2. Tachyphylaxis and/or tolerance - tachyphylactic responses from the use of TSH are not unusual. Stamler and Katz ('51) report a thyrotropic response similar to the one observed in this study. They account for it on the basis of tachyphylaxis.

3. The amount of thyroid substance resulting from T.S.H. activity does not appear to be adequate for the augmentation of epinephrine sclerosis. This appears to be the most acceptable explanation of the thyrotropic responses reported here.

Potassium Iodide

The responses observed when repeated administrations of potassium iodide were given to rabbits receiving an epinephrine sclerogenic regimen are in general contrary to the results of previous reports. It appears evident that repeated epinephrine treatment is able to induce medial arteriopathic changes in the rabbit even in the presence of large quantities of iodide ion. This is contrary to the opinion and reports of Cummins and Stout ('06 - '07), or Strauss ('36). Results of Biland ('06) or Loeb and Fleisher ('07) led these investigators to contend that iodide ion exerts an aggravating influence upon epinephrine lesions and actually augments the sclerogenic response to epinephrine. It appears from the results presented in the present dissertation, that potassium iodide exerts a negligible, or more likely, no effect upon epinephrine induction of medial arteriopathy.

Dinitrophenol

Dinitrophenol did not augment epinephrine sclerosis, in this way differing from thyroxine. No sclerosis was observed even though near toxic levels of dinitrophenol were employed in this study. This is typical of the responses associated with dinitrophenol in that although this drug does cause an increase in general basal metabolic rate, it

exerts no influence upon thyroid iodine, protein bound iodine or upon cholesterol levels in intact organisms (Warner '55). These results, indicate that a generalized increase in basal metabolic rate per se does not necessarily augment arteriosclerogenesis. This finding would lead one to doubt that there is a direct correlation between basal metabolic rate effects of thyroid and its effects upon epinephrine arteriopathy.

Propylthiouracil

The results obtained with propylthiouracil indicate that this substance by itself possesses a definite arteriosclerogenic action. This fact alone though important takes on greater significance in the light of the following experiments.

Previous to the year 1946 atherosclerosis was induced experimentally in the rabbit, chick and guinea pig by means of cholesterol feeding. Atherosclerotic lesions could not be produced experimentally in the dog, cat or other mammals by cholesterol feeding. In 1946 Steiner and Kendall reported an experiment where 3 dogs were fed 0.5 - 1.2 g. thiouracil plus high cholesterol diet. This diet was maintained for a year or more. One animal served as the control for this study and was fed thiouracil plus a usual dog diet. During the feeding period blood cholesterol levels averaging 1000 mg. % were observed and some levels reached over 2000 mg. %. Termination of the experiment revealed extensive atherosclerotic involvement of many blood vessels in the three dogs given cholesterol and thiouracil. It was of interest to note that

the abdominal aortas of these animals were particularly involved. Because of this observation and the fact that medial fibrosis, hyalinization and even calcification was observed to be associated with intimal proliferation, the authors were of the opinion that striking similarities existed between the experimental lesions in the dog and the lesions found in man. Because of the fact that the single control animal did not develop vascular lesions these authors came to the conclusion that thiouracil exerted its sclerogenic effect solely by its antithyroid action, causing a rise in blood cholesterol levels, and this hypercholesterolemia was considered as being the immediate factor leading to atherosclerosis.

Steiner et al ('49) did not observe any lesions developing in dogs put on a thiouracil diet for 14 months. Three animals were used in this study. Dogs placed on a thiouracil-cholesterol diet developed hypercholesterolemia and a distribution of lesions comparable to that occurring in man. Medial involvement was quite pronounced in these animals in that fibrosis, hyalinization and calcification was a common occurrence in these animals. Bevans et al ('51) observed early vascular changes occurring in dogs fed cholesterol and thiouracil. It was observed that lipid first infiltrates the media and that these changes are secondarily followed by intimal proliferation, and further lipid accumulation. No control animals fed thiouracil without cholesterol were used in this study.

In a sequel report to the one presented above, four dogs were placed on a thiouracil-cholesterol diet. Two of the dogs were examined

after 2 months while the other two animals were examined 4 months later. Nine other animals were given cholesterol-thiouracil for a period of 6 months and examined 4 months after being taken off this regimen. Regression of the lesions was a common occurrence. Lipid accumulations gradually decreased but it is of interest to note that the medial degenerative changes persisted long after the loss of lipid.

In yet another work Moses ('54) subjected hypertensive dogs to a high cholesterol diet and either a propylthiouracil or radioiodine intake. It was observed that dogs receiving the propylthiouracil exhibited the greatest amount of aortic arteriopathy. The explanation of the above finding was supposed to be due to a hypercholesterolemia.

It should be pointed out that all of the above investigators are of the opinion that thiouracil or propylthiouracil mediate their atherogenic effects via a hypothyroid influence and a resultant hypercholesterolemia. This is justifiable insofar as the control animals employed did not display any lesions. But it is also quite unusual that such a small group of four control animals, that is, dogs receiving antithyroid substance alone, were used. The possibility exists that an arteriopathy due solely or primarily to the antithyroid drug was not uncovered from lack of sufficient control data. Certainly, the arteriopathy, as displayed in the rabbit experiment reported here is real. This suggests that the comparison wherein the canine experiments are likened to human atherosclerosis, is perhaps not totally valid. Perhaps here a

medial vascular arteriopathy is responsible for or is a predisposing factor in the establishment of such canine "atherosclerosis".

Interesting speculation can arise as a consequence of the results described in this dissertation. The senescence or the toxin theories do not adequately account for the induction of epinephrine-thyroxine medial arteriopathy. It may also be stated that the mechanical trauma theory or hypoxia theory of arteriosclerogenesis, while compatible with the findings in this dissertation, are almost too simple for an explanation of the striking vascular changes involved. The known transitory effects of epinephrine upon blood pressure changes could induce focal damage. Thyroxine, on the other hand, acting continuously could foster and abet this initial medial derangement and as a consequence lead to widespread, extensive arterial degeneration. Thyroxine augmentation of epinephrine medial arteriopathy does not appear to be due to a simple rise in basal metabolic activity nor does this effect appear to reside in the thyroxine molecule itself since similar activity was produced by triiodothyronine. Although this study did not involve investigations of hypertension or hypoxia, the comparatively evanescent nature of these phenomena after epinephrine suggest that these two factors do not provide an adequate explanation of the resulting arteriopathy. The possibility must be considered that thyroxine acts directly upon the cells of the vascular wall.

CHAPTER VII

SUMMARY

1. This study was undertaken to investigate, or if possible, clarify the function of the thyroid gland in the induction of an experimental medial arteriopathy, as it occurs in the rabbit.
2. Administration of thyroxine to animals receiving sclerogenic amounts of epinephrine augments the epinephrine effect, resulting in a severe medial arteriopathy which is primarily observed in the thoracic aorta. A degree of the arteriopathy is also observed in the pulmonary and femoral vessels. Apparently, only large vascular trunks are affected. The small arteries which were examined, the cerebral, coronary or renal did not appear to be affected.
3. Triiodothyronine displays a sclerogenic effect similar to that observed to occur when thyroxine is used in epinephrine sclerosis.
4. Thyrotropin did not significantly alter the sclerogenic effect of epinephrine.
5. Dinitrophenol, even when administered in near toxic amounts, does not appear to augment epinephrine arteriopathy nor does it appear to have any sclerogenic properties itself.
6. Large doses of potassium iodide did not influence epinephrine sclerosis. Statistical analysis did not reveal any augmentary or

inhibitory sclerogenic effect.

7. Removal of the thyroid gland, resulted in complete abolition of the sclerogenic action of epinephrine. This fact coupled with the above observations, and the reported finding of von Balo ('39) and others to the effect that thyroxine displays a sclerogenic potential by itself as well as serving to augment the sclerogenic action of epinephrine, indicates that the thyroid gland is essential for the induction of medial arteriopathy.

8. Propylthiouracil displayed a pronounced sclerogenic response which is attributable to its own properties. It also appears that this substance is able to increase the incidence of epinephrine arteriopathy. Propylthiouracil arteriopathy is characterized by medial scarring which is located adjacent to the intima. The lesions appear to be somewhat different from those of the epinephrine type in that in addition to this juxtaposition to the intima no calcification was observed.

This experiment raises some doubts as to the validity of certain previously reported canine experiments. The dog develops atherosclerosis only when under high cholesterol diet and in the presence of one of the antithyroid substances. It was supposed that the antithyroid agents enhance atherogenesis solely by means of their antithyroid activity which in turn promotes hypercholesterolemia. The hypercholesterolemia was thought to be the primary cause of atherogenesis. These experiments suggest that the sclerogenic effect of the antithyroid agent itself may be a major causative factor in canine "atherosclerosis".

CHAPTER VIII

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PLATE I

EXPLANATION OF FIGURES

Figure 1. Aorta of control rabbit (CCB) treated with physiological saline for a 15 day injection period. Section shows normal intimal and medial components. Hematoxylin and Eosin. 100x

ABBREVIATIONS

i - intima

m - media

Figure 2. Aorta of rabbit (EB 5) receiving 50 micrograms/kg. epinephrine daily for a total of 15 injections. Only minimal aortic damage is present as exemplified by a separation and derangement of the medial components. Hematoxylin and eosin. 100x

ABBREVIATIONS

Same as Figure 1.

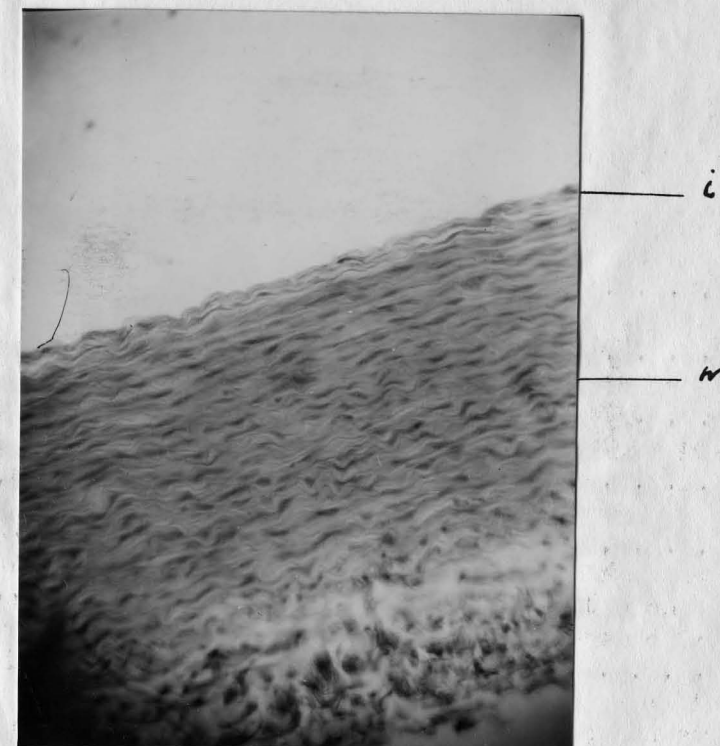


Figure 1

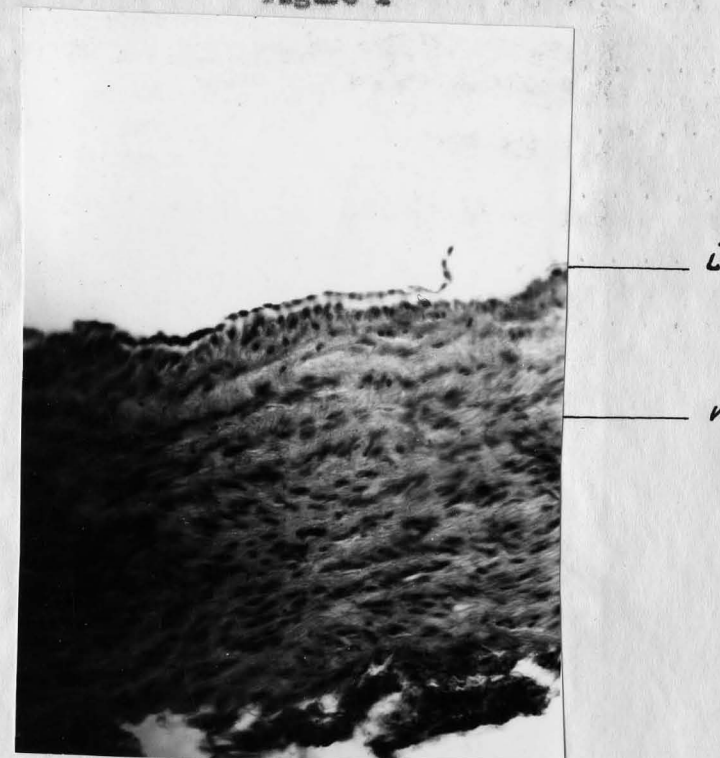


Figure 2

PLATE II

EXPLANATION OF FIGURES

Figure 3. Gross study of longitudinally slit aortae. "A" represents a normal or control vessel while "B" is a sclerosed vessel of a rabbit with 15 daily intravenous injections of epinephrine as in previous figure plus daily 0.15 mg./kg. subcutaneous injections of thyroxin. Note indented areas of calcification within this vessel. \times_2 .

ABBREVIATIONS

c - areas of calcification

Figure 4. Microscopic study of aorta of rabbit (ID) treated in the identical manner as explained in figure 3. Note the band of degenerated tissue running thru the central portion of media. This area appears to consist of calcium-like deposits. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media

c - calcium-like band

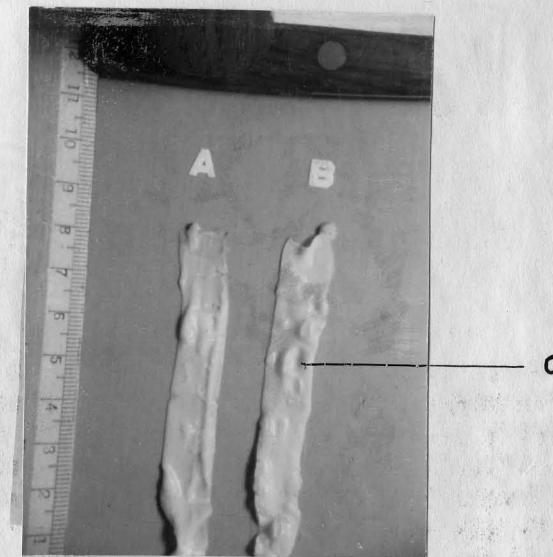


Figure 3

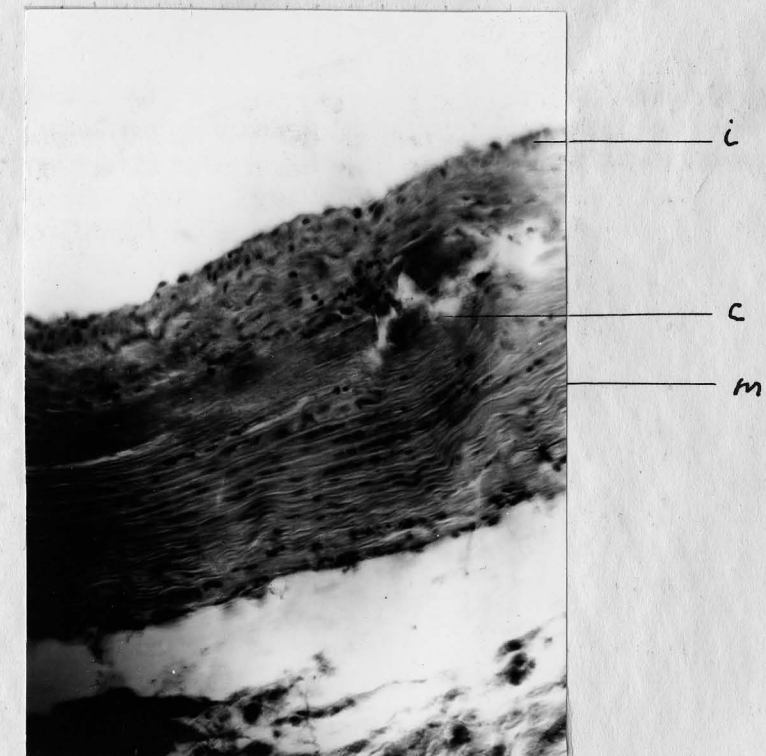


Figure 4

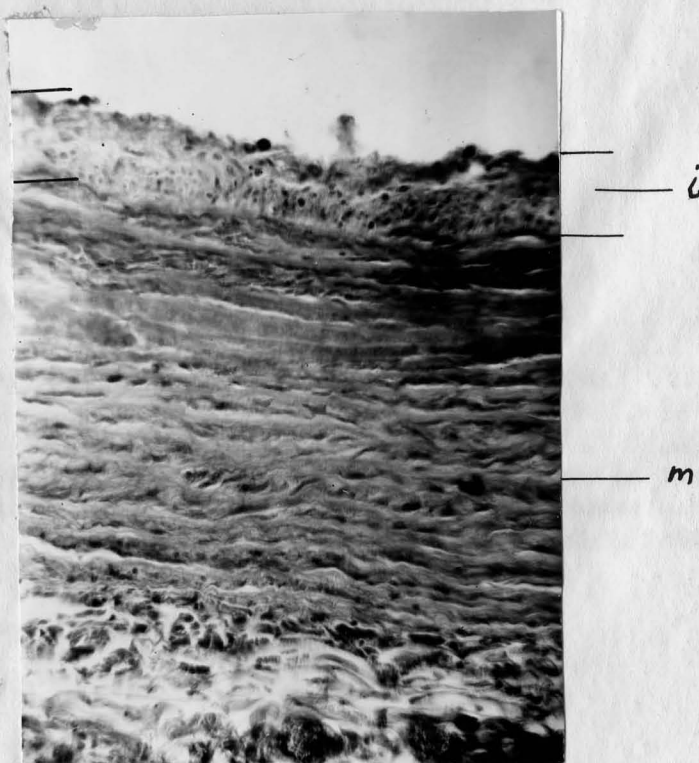


Figure 5

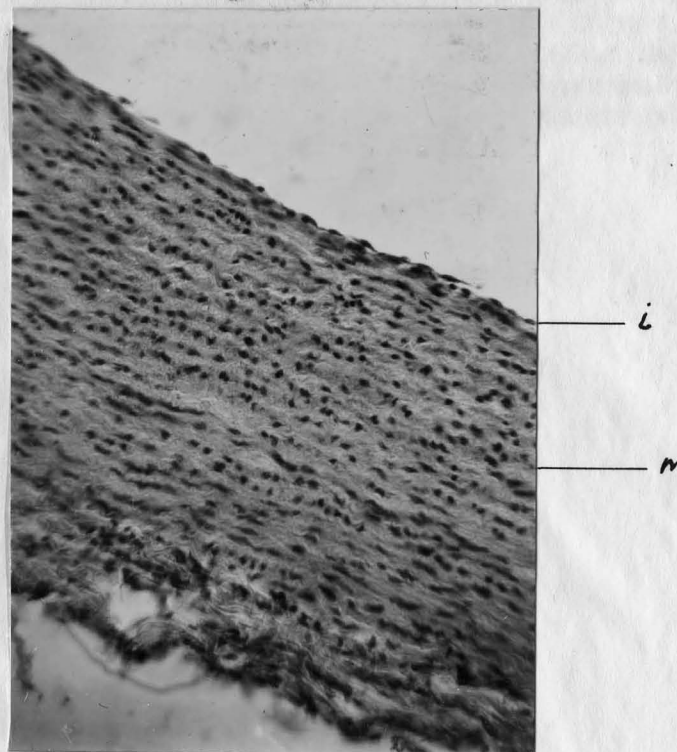


Figure 6

PLATE III

EXPLANATION OF FIGURES

Figure 5. Aorta of animal (IF) treated with epinephrine plus thyroxin as described in figure 3. Maximal intimal proliferation is present which is distinctly greater than that observed in a control animal as shown in figure 2. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media

Figure 6. Pulmonary artery of control rabbit (CCA) treated with intravenous saline. Section shows normal size and cellular structure of intimal and elastic components. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media

PLATE IV

EXPLANATION OF FIGURES

Figure 7. Pulmonary artery of rabbit (III B) treated with the standard epinephrine regimen plus subcutaneous injections of 0.25 mg./kg. thyroxine for the usual 15 day treatment. The artery appears to be hyalinized and devoid of muscular components unlike that observed in the artery shown in figure 6. Little if any hematoxylin was observed in this medial degenerated area. Hematoxylin and eosin. 100x

ABBREVIATIONS

m - media

md - medial area of degeneration

Figure 8. Branch of left pulmonary artery (CCC) in hilus of lung. The vessel was taken from a saline control animal. Normal cellular composition and displacement appears to be present in this vessel. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media



Figure 7

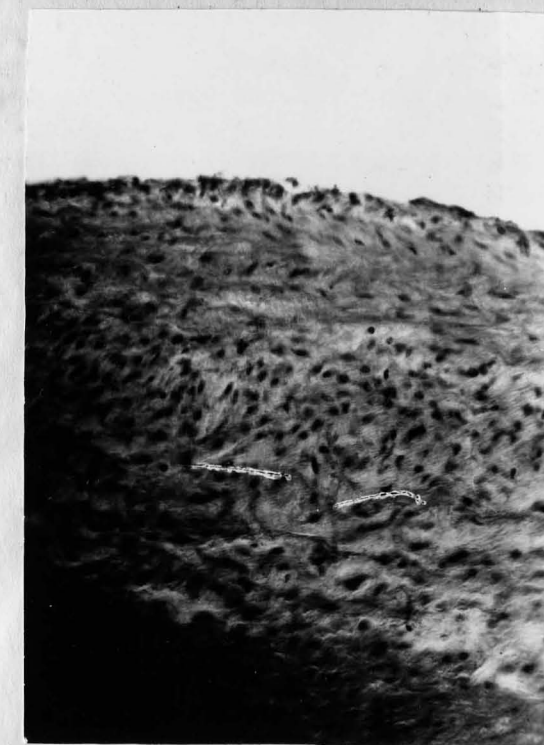


Figure 8

PLATE V

EXPLANATION OF FIGURES

Figure 9. Branch of left pulmonary artery in hilus of lung. This vessel was taken from a rabbit (II I) treated with the same epinephrine-thyroxine procedure as described in figure 3. Medial hyalinization appears to be present. There is also a separation of medial components. Hematoxylin and eosin. 100x

ABBREVIATIONS

m - media

md - area of medial degeneration

Figure 10. Cerebral artery of a saline control animal (CC G). This is a vessel taken from the base of the brain. The intimal and medial cellular components appear to be normal. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media



Figure 9

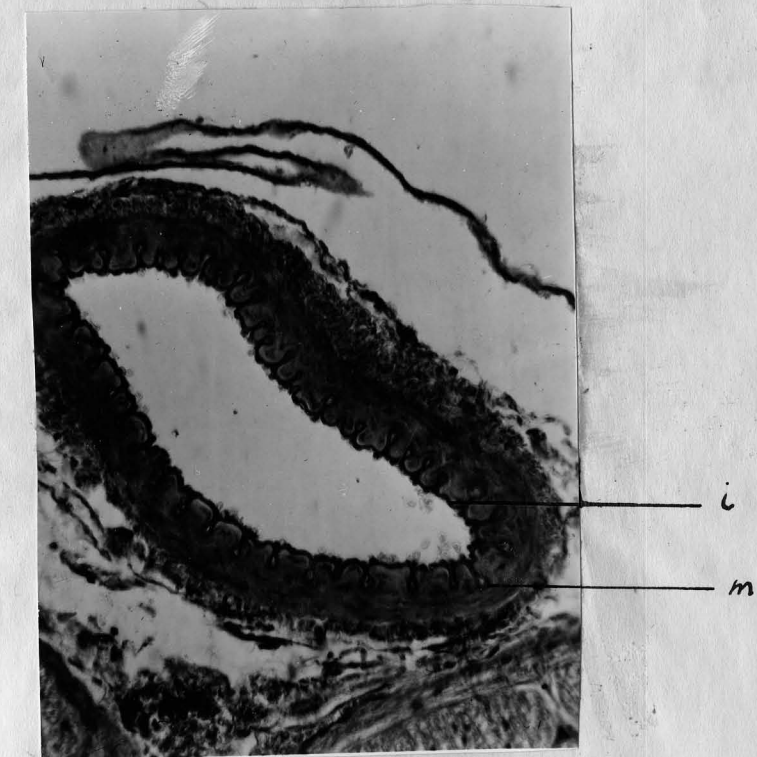


Figure 10

PLATE VI

EXPLANATION OF FIGURE

Figure 11. Cerebral artery taken from base of brain of rabbit (I H) treated with epinephrine plus thyroxine. This regimen is the same as that described for figure 3. The cellular components appear to be in a normal state and it is apparent that there is no essential difference between this vessel and the vessel taken from the control animal represented in figure 10. Hematoxylin and eosin. 100x

ABBREVIATIONS

Same as in figure 10

Figure 12. Coronary artery of a saline control animal (CC A). The intimal and medial cellular components appear to be in a normal state. Hematoxylin and eosin. 100x.

ABBREVIATIONS

i - intima

m - media

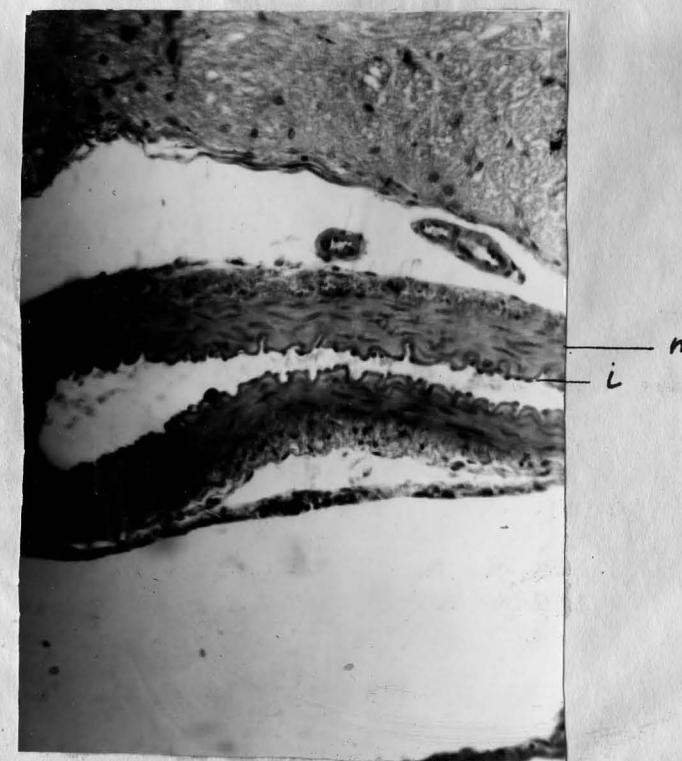


Figure 11

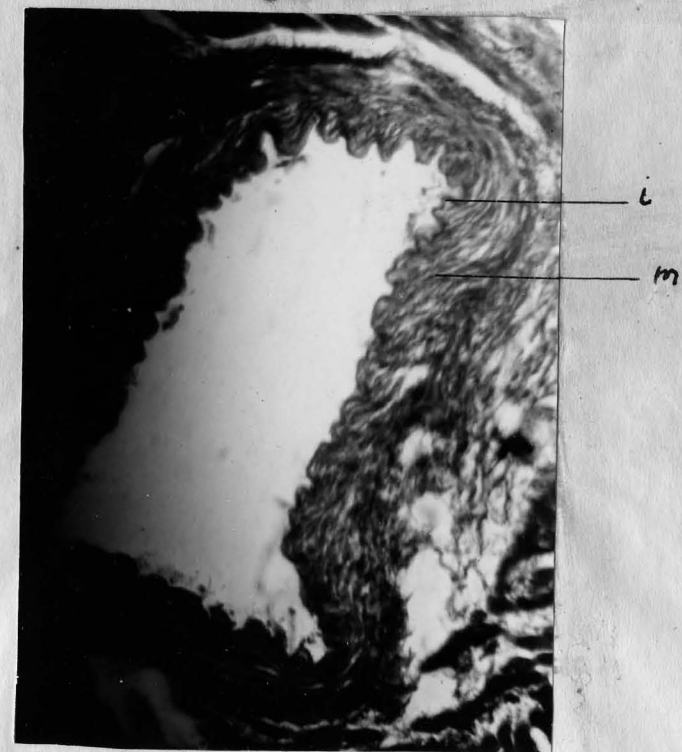


Figure 12

PLATE VII

EXPLANATION OF FIGURES

Figure 13. Coronary artery of rabbit (I I) treated with the epinephrine-thyroxine regimen described in figure 3. Essentially there is no difference between this vessel and the vessel of the saline control animal pictured in figure 12. Hematoxylin and eosin. 100x

ABBREVIATIONS

Same as in figure 12.

Figure 14. Coronary artery of a saline control rabbit (CC D). This vessel is stained with orcein connective tissue stain as well as eosin and hematoxylin. It appears that the elastic tissue fibers are sparsely settled. This indicates a normal rabbit artery condition. 100x

ABBREVIATIONS

in - internal elastic membrane

m - media



Figure 13



Figure 14

PLATE VIII

EXPLANATION OF FIGURES

Figure 15. Left femoral artery of a saline control animal (CC B). The medial and intimal cellular components appear to be in a normal state. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media

Figure 16. Left femoral artery of a rabbit (I G) treated with epinephrine and thyroxine in the manner described under figure 3. There appears to be medial hyalinization. This portion of the vessel appears to be most severely involved. Hematoxylin and eosin. 100x

ABBREVIATIONS

m - media

md - medially degenerated area

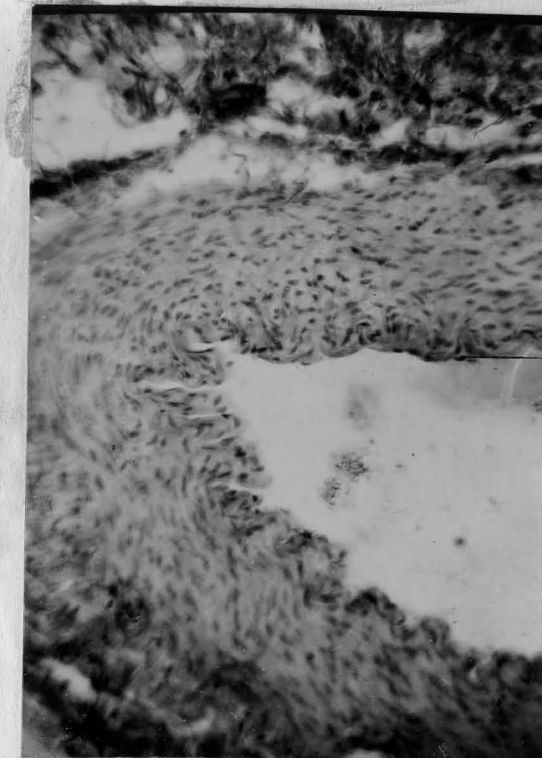


Figure 15

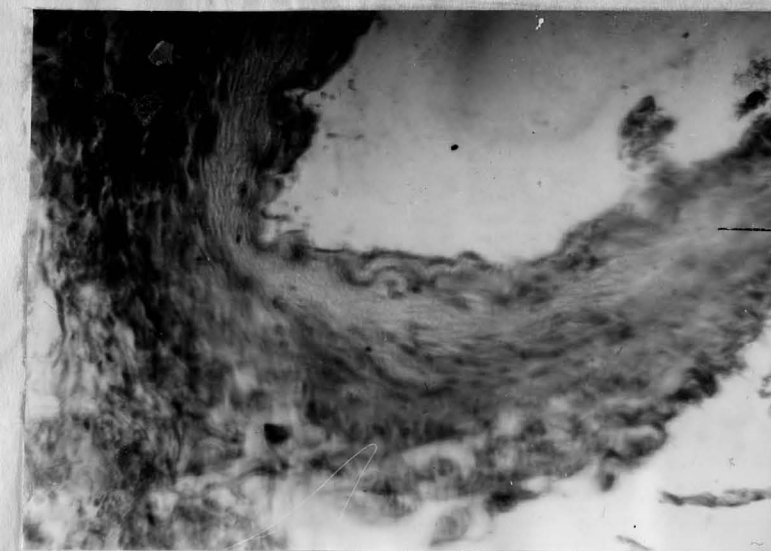


Figure 16

PLATE IX

EXPLANATION OF FIGURES

Figure 17. Aorta of animal (XVI H) treated with intravenous injections of 50 micrograms/kg. epinephrine plus 0.05 mg./kg. triiodothyronine. These doses were given daily for a total of 15 injections. The medial area is necrotic and there appears to be a shattering of this calcium-like mass. Hematoxylin and eosin. 100x

ABBREVIATIONS

m - media

md - area of medial necrosis

Figure 18. Aorta of rabbit (XII E) treated with the above epinephrine regimen plus 0.01 unit T.S.H./kg. intravenously for a total of 15 days. Only minimal aortic damage can be observed with a separation and disorientation of the medial cellular components. Comparison of this section with that shown in figure 2 shows essentially no real difference. Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media



Figure 17

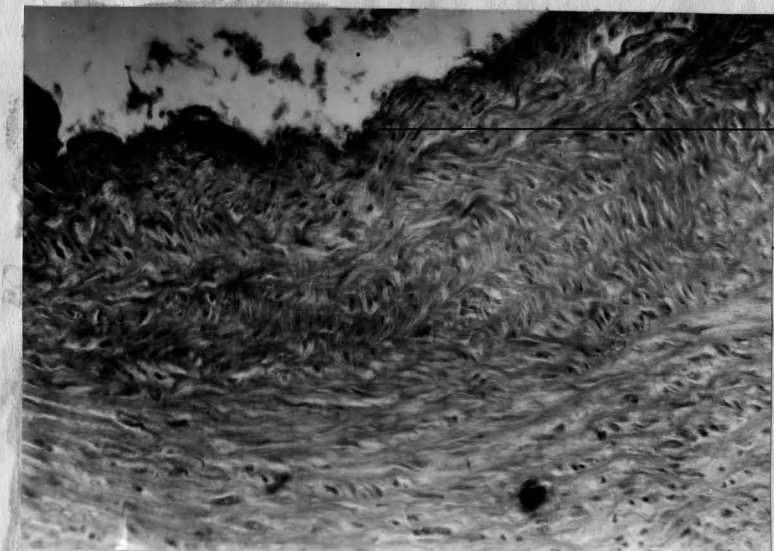


Figure 18

PLATE X

EXPLANATION OF FIGURES

Figure 19. Aorta of rabbit (VII F) treated with epinephrine plus 35 mg./kg. D.N.P. subcutaneously for a total of 15 injections. The degenerative changes are similar if not identical to those present in the epinephrine treated group as displayed in figure 2. Hematoxylin and eosin. 100x

ABBREVIATIONS

Same as figure 18

Figure 20. Aorta of rabbit (XI C) administered the standard epinephrine treatment plus 500 mg./kg. potassium iodide orally, 3 times per week for a total of 15 days. The medial derangement appears to be quite similar to that observed in epinephrine treated animals as shown in figure 2. Hematoxylin and eosin. 100x

ABBREVIATIONS

Same as figure 18.

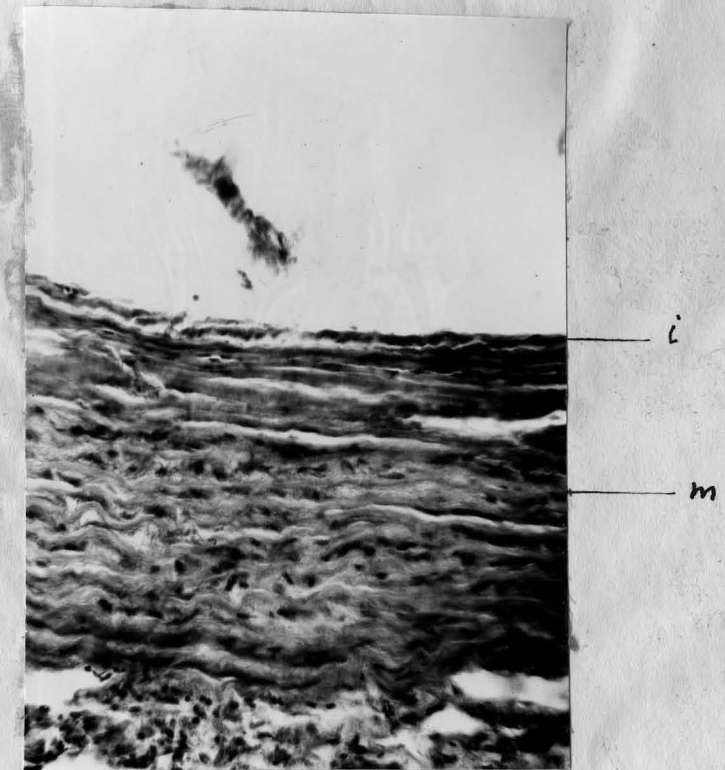


Figure 19

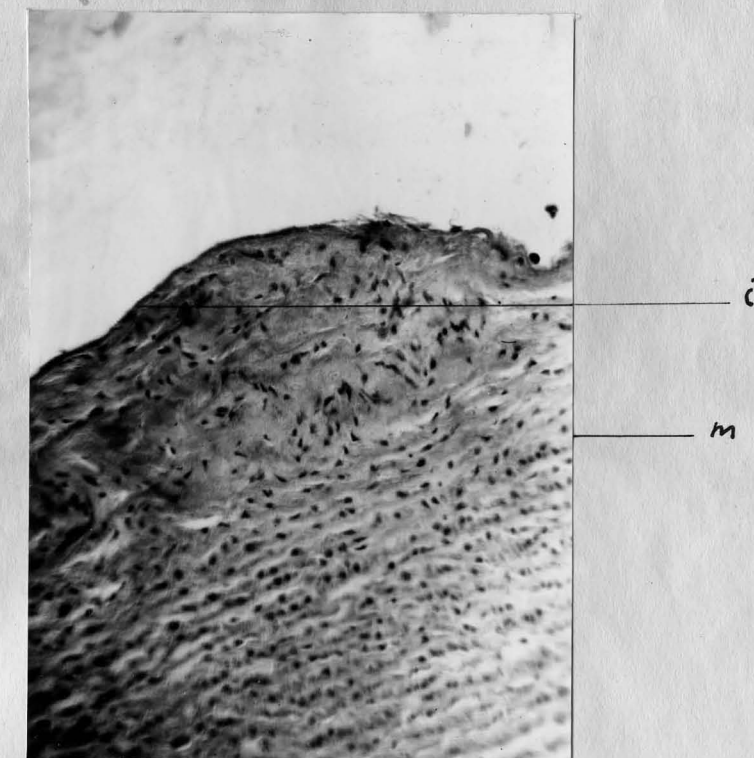


Figure 20

PLATE XI

EXPLANATION OF FIGURES

Figure 21. Aorta of thyroidectomized animal (VIII O) treated with the standard epinephrine regimen. This vessel appears to display normal cellular constituents. Comparison of this section and a section of aorta taken from a control rabbit treated with saline reveals essentially no difference. (Figure 1 displays an aorta of a control animal.) Hematoxylin and eosin. 100x

ABBREVIATIONS

i - intima

m - media

Figure 22. Aorta of rabbit (XV J) treated with the standard epinephrine regimen together with the administration of 125 mg./kg. propylthiouracil orally for a total of 15 days. Aortic damage appears to be rather severe. There is a definite difference between animals treated in this manner and those treated with epinephrine alone (figure 2) in that there is a great separation of the elastic fibers. The elastic elements appeared to be wavy and elongated. This derangement appears to be adjacent to the intima while that observed to occur from epinephrine appears to be deeper in the media. Hematoxylin and eosin. 100x

ABBREVIATIONS

e - elastic folds

m - media

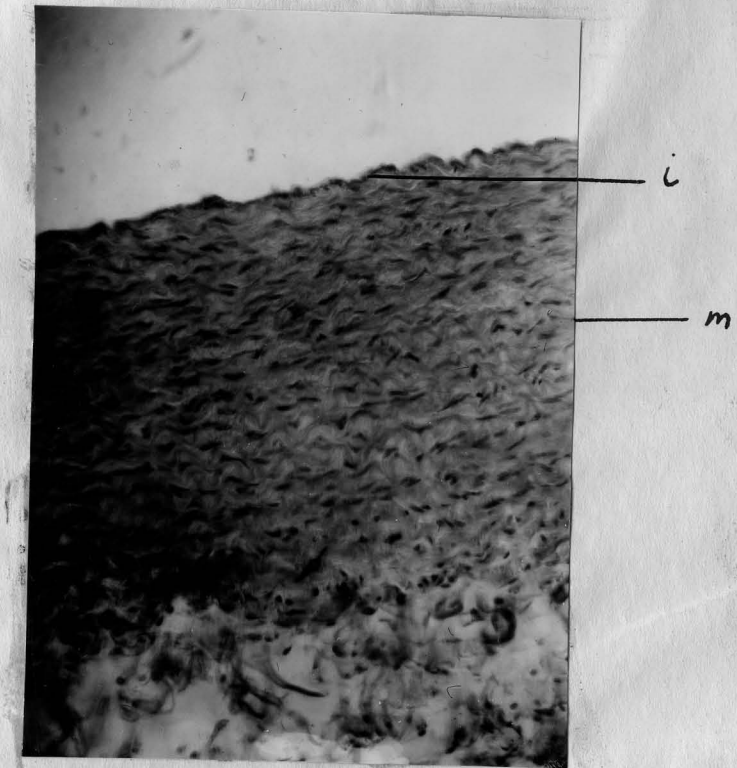


Figure 21

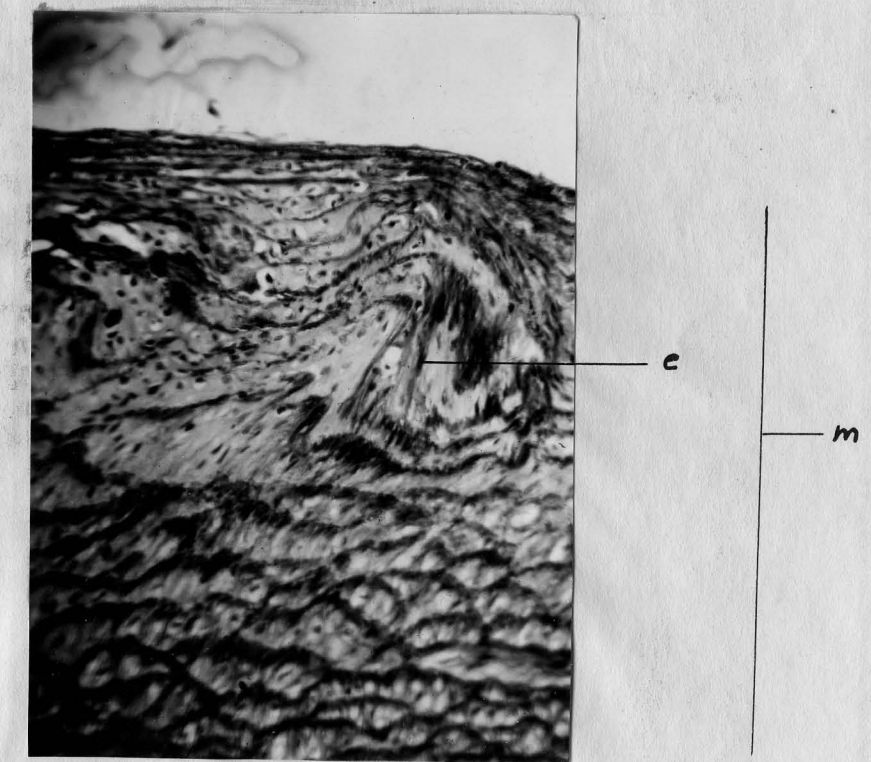


Figure 22

PLATE XII

EXPLANATION OF FIGURES

Figure 23. Thyroid gland of an untreated stock rabbit. The thyroid epithelium is cuboidal and appears to be in a normal state. Hematoxylin and eosin. 100x

ABBREVIATIONS

e - epithelium

c - colloid follicles

Figure 24. Thyroid gland of rabbit (XV H) treated with epinephrine plus propylthiouracil as described in figure 22. The epithelium appears to be in a normal state. Essentially there is very little, if any, difference between this section and the one of the control animal shown above. Hematoxylin and eosin. 100x

ABBREVIATIONS

e - epithelium

c - colloid follicles

v - vacuoles

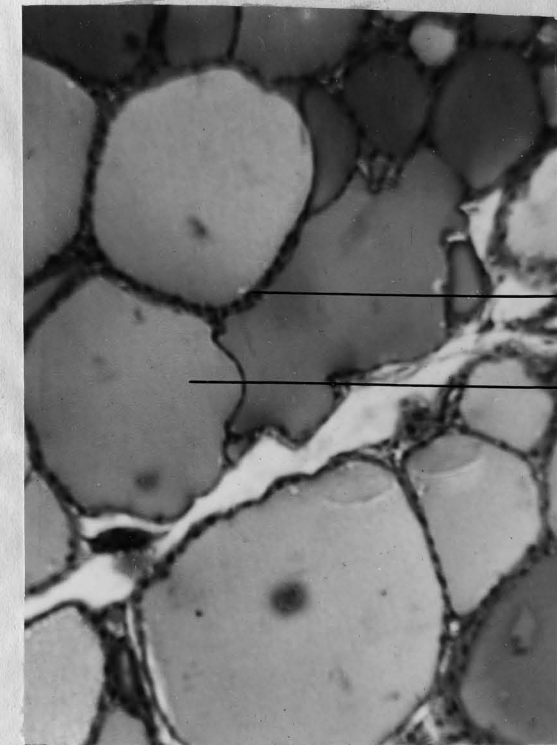


Figure 23

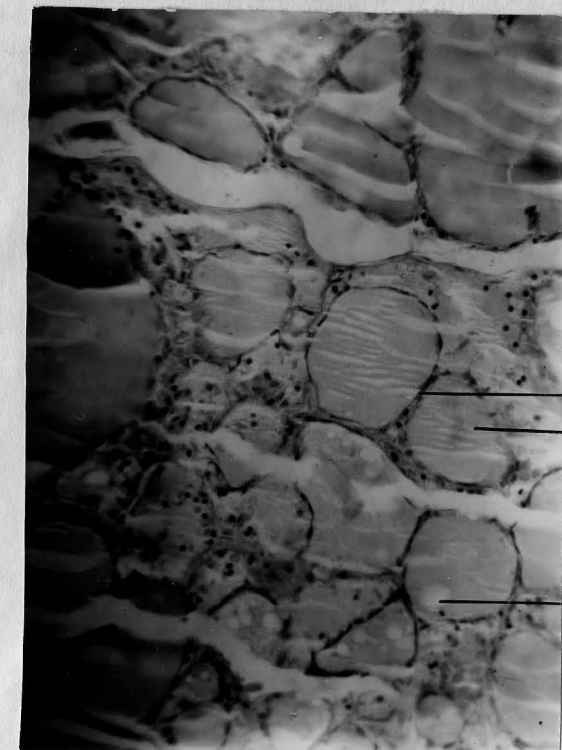


Figure 24

PLATE XIII

EXPLANATION OF FIGURES

Figure 25. Aorta of rabbit administered (CC B) saline for a 15 day period. This vessel appears to be in a normal state with a typical distribution of elastic elements. Iron hematoxylin. 100x

ABBREVIATIONS

m - media

e - elastic fibers

Figure 26. Aorta of rabbit (XVII B) given 15 daily, oral administrations of 125 mg./kg. propylthiouracil. The elastic elements adjacent to the intima appear to be separated. Medial scarring is also quite evident indicating a pathological state as compared to the above section. Iron hematoxylin. 100x

ABBREVIATIONS

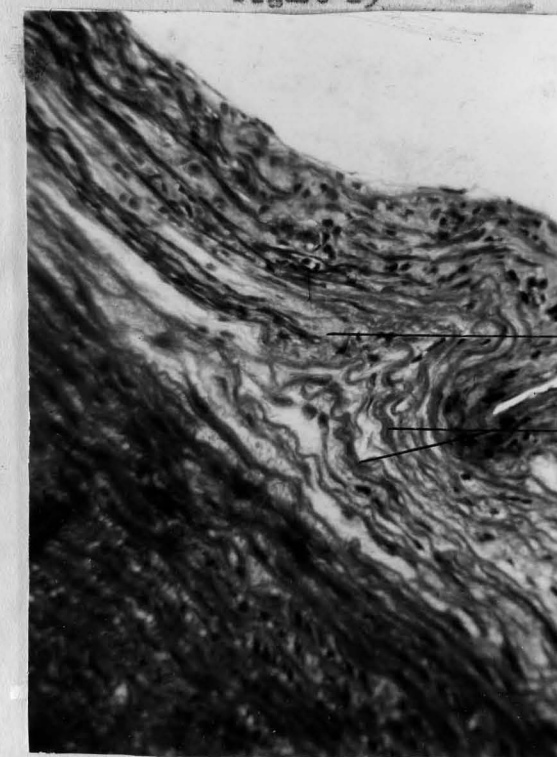
e - elastic fibers

a - area of medial scarring



e
m

Figure 25



a
e

Figure 26

PLATE XIV

EXPLANATION OF FIGURES

Figure 27. Thyroid gland of normal stock rabbit stained with iron hematoxylin. The epithelium is of the cuboidal type indicating a normal state. The colloid is gray in color indicating a normal stored colloid state. 100x

ABBREVIATIONS

e - thyroid epithelium

c - colloid

Figure 28. Thyroid gland of rabbit (XVII I) receiving propyl-thiouracil according to the procedure described in figure 26. There apparently is no difference between this section and the normal one shown above indicating a normal state. Iron hematoxylin. 100x

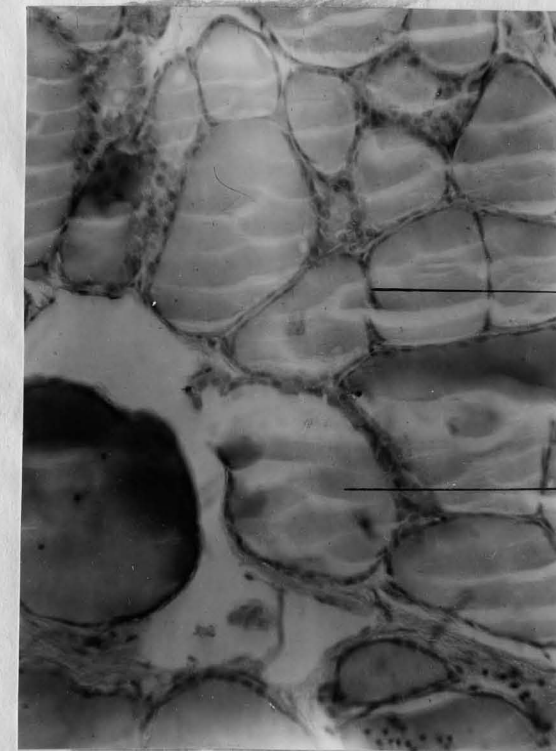


Figure 27

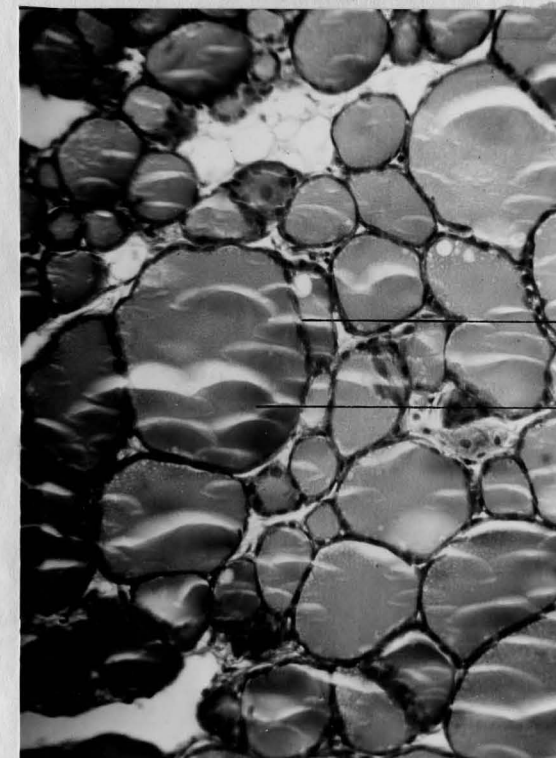


Figure 28

PLATE XV

EXPLANATION OF FIGURES

- Figure 29. Aorta of saline control rabbit. The vessel appears to be in a normal state. Orcein and hematoxylin. 100x
- Figure 30. Aorta of rabbit (XVII D) receiving propylthiouracil according to the procedure described in figure 26. A large medial scar is present in an area adjacent to the intima. The disposition of the elastic fibers is similar to that observed in the vessel shown in figure 26. Thus another staining procedure displays essentially the same deranged state. Orcein and hematoxylin. 100x

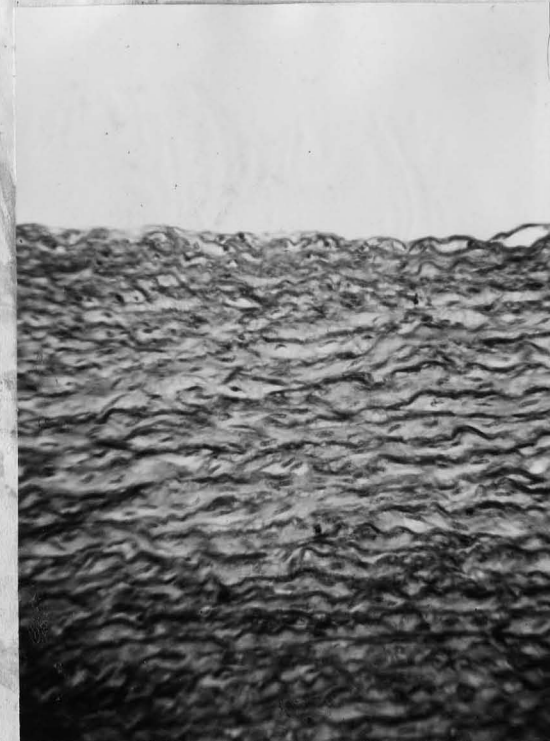


Figure 29

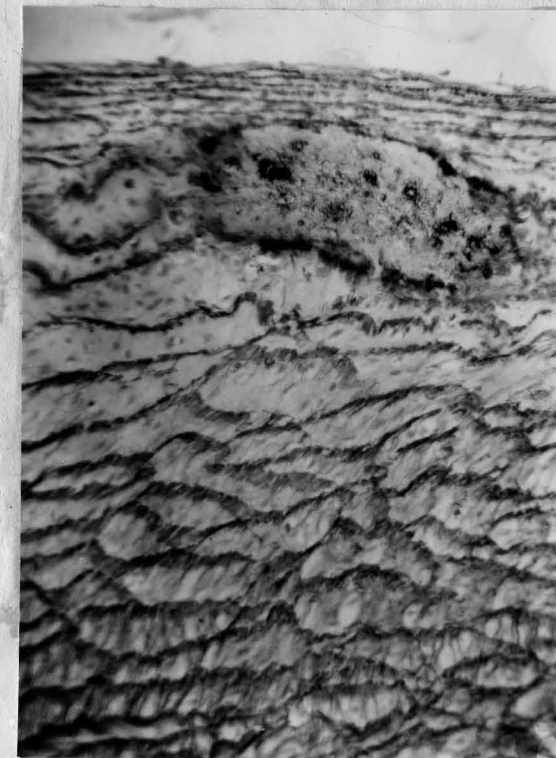


Figure 30